METHOD AND DETECTOR FOR IDENTIFYING SUBTYPES OF HUMAN PAPILLOMA VIRUSES

FIELD OF THE INVENTION

[0001] The present invention relates to a method and a detector for detecting human papilloma viruses, and more particularly to a method and a detector for simultaneously detecting and identifying subtype of human papilloma viruses (HPV).

BACKGROUND OF THE INVENTION

[0002] In humans, more than 70 genetically distinct strains of human papilloma virus (HPV) have been identified based on DNA hybridization studies. According to some reports, different HPV types cause distinct diseases. For example, "Low-risk" HPVs, e.g., HPV 6 and HPV 11, cause benign hyperplasias such as genital warts, while "high-risk" HPVs, e.g., HPV-16, HPV-18, HPV-31, HPV-33, HPV-54, and the like, can cause cancers such as cervical or penile carcinoma.

[0003] Cervical cancer is the most common cancer in women. The consorts are often men with penile warts. Sexual activity appears to be an important predisposing factor of the epidemic disease and precancerous lesions. In early 5 to 10 years during the development of cervical cancer, cervical cells form cervical intraepithelial neoplasm.

[0004] Recently, in order to decrease the incidence of cervical cancer, Pap smear is used for the cervical cancer screening. However, the Pap smear has a false negative rate of about 30%~40%. In addition, it is known that more that 95% of cervical carcinoma tissue contain detectable DNA sequences for known varieties of the human papilloma virus (HPV). Hence, the combination of Pap

smear and HPV detection for the cervical cancer screening is necessarily considered.

[0005] The Applicant cooperates with the hospital to do the epidemiological research in women cervical cancer by using Pap smear and HPV detection, wherein the HPV detection is proceeded by using polymerase chain reaction and nucleotide sequencing. There are 2424 women aged from 16 to 84 for the epidemiology research, wherein 1963 women provide the effective specimen. The research results are shown as follows.

1) 1.9% (37/1963) of the women have abnormal cytological smears.
2) 12.7% (244/1926) of the women with normal cytological smears but have HPV infection.
3) The HPV prevalence in the women with abnormal cytological smears is 51.4% (19/37) and positively relative to the degree of the abnormal cytological smears, wherein the incidence of abnormal non-typical squamous cells is 23.1%, the incidence of low abnormal epithelial cells is 41.7%, and the incidence of high abnormal epithelial cells is 75%.
4) The subtypes of human papilloma viruses detected in the specimens are HPV 52, HPV 58, HPV 70, HPV 16, HPV 18, HPV 68, HPV 33, HPV 66, HPV 35, HPV 37, HPV 54, HPV 59, HPV 67, HPV 72, HPV 69, HPV 82, HPV 39, HPV 31, HPV 32, HPV HLT7474-S, HPV 6, HPV CP8061, HPV 62, HPV CP8304, HPV 44, HPV 11, HPV 61, HPV 74, HPV 42 and HPV 43.

[0006] The conventional HPV detecting kits are only used for detecting 18 subtypes of human papilloma viruses including high risk HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV

58, HPV 59 and HPV 68, and detecting low risk HPV 6, HPV 11, HPV 42, HPV 43 and HPV 44.

[0007] However, according to the comparison of the epidemiology research and the conventional HPV detecting kits, several clinically-important subtypes of human papilloma viruses contained in a specimen could not be identified by the conventional HPV detecting kits. In addition, the conventional HPV detecting kits only tell the information of HPVs contained in a specimen by two categories, high risk HPVs or low HPVs, rather than tell the definite subtypes as which they are classified. Therefore, except the high risk HPVs and the low risk HPVs, if other HPV subtypes are contained in the specimen, the conventional HPV detecting kits can not identify immediately, which would seriously affects the diagnosis accuracy. Furthermore, the conventional HPV detecting kits lack the system control for checking the house-keeping genes contained in a specimen. Without the system control, it will be hard to confirm whether the detecting protocols are precisely followed. That is, the user can not tell the positive/negative result comes from the HPV subtypes presence/absence or comes from the incorrect protocols execution. Therefore, the conventional detecting kit without the system control would not be able to provide a convincing result.

[0008] From the above description, it is known that the conventional detecting kit can not identify many HPV subtypes at the same time and it does not include an internal control in the detecting system. Therefore, how to simultaneously detect many HPV subtypes contained in a biological simple and design an accurate internal control in the detecting kits have become a major problem waited to be solved. In order to overcome the foresaid drawbacks of the conventional HPV detecting kits, the present invention provides a method

and a detector for simultaneously detecting and identifying subtypes of human papilloma viruses contained in a sample.

SUMMARY OF THE INVENTION

[0009] It is therefore an object of the present invention to provide a detector for simultaneously detecting and identifying subtypes of human papilloma viruses (HPV) contained in a sample.

[0010] The main purpose of the present invention is to provide a HPV detecting kit, which is able to diagnose multiple HPV subtypes (up to 39 different subtypes) at the same time, allowing the rapid and reliable detection and identification of HPV possibly present in a biological sample.

[0011] It is another object of the present invention to provide a rapid and reliable method to detect and identify the HPV present in a biological sample.

[0012] It is another object of the present invention to provide a HPV detecting kit with high specificity and accuracy, which includes an internal control to show whether the detecting process is well handled so that the detecting result is dependable.

[0013] It is another object of the present invention to provide a number of oligonucleotides as probes for detecting and identifying the HPV present in a biological sample.

[0014] According to one aspect of the present invention, a detector for detecting and simultaneously diagnosing at least one subtype of human papilloma viruses (HPV) contained in a biological sample, comprises: a carrier, a plurality of micro-dots immobilized on the carrier, wherein each micro-dot is for identifying one particular HPV subtype, and the HPV subtype is one selected from a group consisting of (HPV 6, HPV 11, HPV 16, HPV 18, HPV 26, HPV 31, HPV 32, HPV 33, HPV 35, HPV 37, HPV 39, HPV 42, HPV 43, HPV 44,

HPV 45, HPV 51, HPV 52, HPV 53, HPV 54, HPV 55, HPV 56, HPV 58, HPV 59, HPV 61, HPV 62, HPV 66, HPV 67, HPV 68, HPV 69, HPV 70, HPV 72, HPV 74, HPV 82, HPV CP8061, HPV CP8034, HPV L1AE5, HPV MM4, HPV MM7 and HPV MM8); and at least one oligonucleotide sequence contained in each the micro-dot that is specific to the one particular HPV subtype, wherein the at least one oligonucleotide sequence serves as a detection probe that hybridizes specifically with an L1 gene sequence of the one particular HPV subtype to form a hybridization complex as a detection indicator, so that each micro-dot identifies one particular HPV subtype via a corresponding oligonucleotide of the one particular HPV subtype, and thereby detecting and simultaneously identifying subtypes of human papilloma viruses.

[0015] In accordance with the present invention, the at least one oligonucleotide that hybridizes specifically with an L1 gene sequence of the one particular HPV subtype is respectively chosen from the following list for each HPV subtype: (SEQ ID NO:1-SEQ ID NO:12) for HPV 6, (SEQ ID NO:13-SEQ ID NO:24) for HPV 11, (SEQ ID NO:25-SEQ ID NO:36) for HPV 16, (SEQ ID NO:37-SEQ ID NO:48) for HPV 18, (SEQ ID NO:49-SEQ ID NO:58) for HPV 26, (SEQ ID NO:59-SEQ ID NO:68) for HPV 31, (SEQ ID NO:69-SEQ ID NO:79) for HPV 32, (SEQ ID NO:80-SEQ ID NO:90) for HPV 33, (SEQ ID NO:91-SEQ ID NO:100) for HPV 35, (SEQ ID NO:101-SEQ ID NO:112) for HPV 37, (SEQ ID NO:113-SEQ ID NO:123) for HPV 39, (SEQ ID NO:124-SEQ ID NO:133) for HPV 42, (SEQ ID NO:134-SEQ ID NO:143) for HPV 43, (SEQ ID NO:144-SEQ ID NO:154) for HPV 44, (SEQ ID NO:155-SEQ ID NO:165) for HPV 45, (SEQ ID NO:166-SEQ ID NO:177) for HPV 51, (SEQ ID NO:178-SEQ ID NO:189) for HPV 52, (SEQ ID NO:190-SEQ ID NO:199) for HPV 53, (SEQ ID NO:200-SEQ ID NO:209) for

HPV 54, (SEQ ID NO:210-SEQ ID NO:218) for HPV 55, (SEQ ID NO:219-SEQ ID NO:228) for HPV 56, (SEQ ID NO:229-SEQ ID NO:239) for HPV 58, (SEQ ID NO:240-SEQ ID NO:250) for HPV 59, (SEQ ID NO:251-SEQ ID NO:261) for HPV 61, (SEQ ID NO:262-SEQ ID NO:272) for HPV 62, (SEQ ID NO:273-SEQ ID NO:283) for HPV 66, (SEQ ID NO:284-SEQ ID NO:294) for HPV 67, (SEQ ID NO:295-SEQ ID NO:305) for HPV 68, (SEQ ID NO:306-SEQ ID NO:316) for HPV 69, (SEQ ID NO:317-SEQ ID NO:328) for HPV 70, (SEQ ID NO:329-SEQ ID NO:341) for HPV 72, (SEQ ID NO:342-SEQ ID .NO:353) for HPV 74, (SEQ ID NO:354-SEQ ID NO:362) for HPV 82, (SEQ ID NO:363-SEQ ID NO:374) for HPV CP8061, (SEQ ID NO:375-SEQ ID NO:386) for HPV CP8034, (SEQ ID NO:387-SEQ ID NO:397) for HPV L1AE5, (SEQ ID NO:398-SEQ ID NO:408) for HPV MM4, (SEQ ID NO:409-SEQ ID NO:419) for HPV MM7, and (SEQ ID NO:420-SEQ ID NO:429) for HPV MM8.

[0016] Preferably, the carrier is a nylon membrane..

[0017] Preferably, the carrier is a glass plate.

[0018] Preferably, the detector is an oligonucleotide biochip.

[0019] Preferably, the at least one oligonucleotide has a length between 15-30 bases.

[0020] Preferably, the detector further comprises a micro-dot containing a Glutaldehyde-3-phosphodehydrogenase (GAPDH) gene, which is used as an internal control.

[0021] According to another aspect of the present invention, a method for detecting and simultaneously diagnosing at least one subtype of human papilloma viruses (HPV) contained in a biological sample is provided. The detecting method comprises steps of: amplifying an L1 gene fragment of human

papilloma viruses (HPV) contained in the biological sample and obtaining an amplification product by polymerase chain reaction (PCR) using primers labeled with signaling substance; hybridizing the amplification product with a detector according to Claim 1 to form a hybridization complex; removing nonhybridized the amplification product; and detecting the hybridization complex through detecting the signaling substance, thereby detecting and simultaneously identifying HPV subtypes contained in the biological sample.

[0022] Preferably, the amplification product has a length of 450 base pairs by using MY09 as sense primer and MY11 as anti-sense primer in polymerase chain reaction (PCR).

[0023] Preferably, the amplification product has a length of 190 base pairs by using MY11 as sense primer and GP6+ as anti-sense primer in polymerase chain reaction (PCR).

[0024] Preferably, the signaling substance is biotin.

[0025] Preferably, the biotin reacts with avidin-alkalinephosphatase to show the hybridization result by presenting a particular color.

[0026] Preferably, the signaling substance is a fluorescent substance.

[0027] Preferably, the fluorescent substance is Cyanine 5.

[0028] According to another aspect of the present invention, a probe which hybridizes to nucleic acid from an HPV subtype, the probe being selected from the group consisting of: SEQ ID NO:1-SEQ ID NO:12 and sequences fully complementary thereto, which hybridize with HPV 6; SEQ ID NO:13-SEQ ID NO:24 and sequences fully complementary thereto, which hybridize with HPV 11; SEQ ID NO:25-SEQ ID NO:36 and sequences fully complementary thereto, which hybridize with HPV 16; SEQ ID NO:37-SEQ ID NO:48 and sequences fully complementary thereto, which hybridize with HPV 18; SEQ ID

NO:49-SEQ ID NO:58 and sequences fully complementary thereto, which hybridize with HPV 26; SEQ ID NO:59-SEQ ID NO:68 and sequences fully complementary thereto, which hybridize with HPV 31; SEQ ID NO:69-SEQ ID NO:79 and sequences fully complementary thereto, which hybridize with HPV 32; SEQ ID NO:80-SEQ ID NO:90 and sequences fully complementary thereto, which hybridize with HPV 33; SEQ ID NO:91-SEQ ID NO:100 and sequences fully complementary thereto, which hybridize with HPV 35; SEQ ID NO:101-SEQ ID NO:112 and sequences fully complementary thereto, which hybridize with HPV 37; SEQ ID NO:113-SEQ ID NO:123 and sequences fully complementary thereto, which hybridize with HPV 39; SEQ ID NO:124-SEQ ID NO:133 and sequences fully complementary thereto, which hybridize with HPV 42; SEQ ID NO:134-SEQ ID NO:143 and sequences fully complementary thereto, which hybridize with HPV 43; SEQ ID NO:144-SEQ ID NO:154 and sequences fully complementary thereto, which hybridize with HPV 44; SEQ ID NO:155-SEQ ID NO:165 and sequences fully complementary thereto, which hybridize with HPV 45; SEQ ID NO:166-SEQ ID NO:177 and sequences fully complementary thereto, which hybridize with HPV 51; SEQ ID NO:178-SEQ ID NO:189 and sequences fully complementary thereto, which hybridize with HPV 52; SEQ ID NO:190-SEQ ID NO:199 and sequences fully complementary thereto, which hybridize with HPV 53; SEQ ID NO:200-SEQ ID NO:209 and sequences fully complementary thereto, which hybridize with HPV 54; SEQ ID NO:210-SEQ ID NO:218 and sequences fully complementary thereto, which hybridize with HPV 55; SEQ ID NO:219-SEQ ID NO:228 and sequences fully complementary thereto, which hybridize with HPV 56; SEQ ID NO:229-SEQ ID NO:239 and sequences fully complementary thereto, which hybridize with HPV 58; SEQ ID NO:240-SEQ ID NO:250 and sequences fully complementary

thereto, which hybridize with HPV 59; SEQ ID NO:251-SEQ ID NO:261 and sequences fully complementary thereto, which hybridize with HPV 61; SEQ ID NO:262-SEQ ID NO:272 and sequences fully complementary thereto, which hybridize with HPV 62; SEQ ID NO:273-SEQ ID NO:283 and sequences fully complementary thereto, which hybridize with HPV 66; SEQ ID NO:284-SEQ ID NO:294 and sequences fully complementary thereto, which hybridize with HPV 67; SEQ ID NO:295-SEQ ID NO:305 and sequences fully complementary thereto, which hybridize with HPV 68; SEQ ID NO:306-SEQ ID NO:316 and sequences fully complementary thereto, which hybridize with HPV 69; SEQ ID NO:317-SEQ ID NO:328 and sequences fully complementary thereto, which hybridize with HPV 70; SEQ ID NO:329-SEQ ID NO:341and sequences fully complementary thereto, which hybridize with HPV 72; SEQ ID NO:342-SEQ ID NO:353 and sequences fully complementary thereto, which hybridize with HPV 74; SEQ ID NO:354-SEQ ID NO:362 and sequences fully complementary thereto, which hybridize with HPV 82; SEQ ID NO:363-SEQ ID NO:374 and sequences fully complementary thereto, which hybridize with HPV CP8061; SEQ ID NO:375-SEQ ID NO:386 and sequences fully complementary thereto, which hybridize with HPV CP8034; SEQ ID NO:387-SEQ ID NO:397 and sequences fully complementary thereto, which hybridize with HPV L1AE5; SEQ ID NO:398-SEQ ID NO:408 and sequences fully complementary thereto, which hybridize with HPV MM4; SEQ ID NO:409-SEQ ID NO:419 and sequences fully complementary thereto, which hybridize with HPV MM7; and SEQ ID NO:420-SEQ ID NO:429 and sequences fully complementary thereto, which hybridize with HPV MM8.

[0029] The foregoing and other features and advantages of the present invention will be more clearly understood through the following descriptions with reference to the drawings, wherein:

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] Fig. 1 is a schematic view showing the detector according to a preferred embodiment of the present invention;

[0031] Fig. 2(a) is a schematic view showing the detector according to a preferred embodiment of the present invention;

[0032] Fig. 2(b) is a schematic view illustrating the subtype of human papilloma viruses identified by each dot shown in Fig. 2(a);

[0033] Fig. 3(a) is the electrophoresis result showing the analyzed PCR products using primer set MY09/MY11 according to a preferred embodiment of the present invention;

[0034] Fig. 3(b) is the electrophoresis result showing the analyzed PCR products using primer set MY11/GP6+ according to a preferred embodiment of the present invention;

[0035] Fig. 3(c) is the electrophoresis result showing the analyzed PCR products using GAPDH primer set according to a preferred embodiment of the present invention;

[0036] Fig. 4(a) is the detecting result on the detector of detecting the PCR products using primer set MY09/MY11 of HPV positive clones according to a preferred embodiment of the present invention;

[0037] Fig. 4(b) is detecting result on the detector of detecting the PCR products using primer set MY11/GP6+ of HPV positive clones according to a preferred embodiment of the present invention;

[0038] Fig. 5 is a view showing the detecting result on the detectors of detecting samples according to a preferred embodiment of the present invention;

[0039] Fig. 6(a) is a schematic view showing the detector according to another preferred embodiment of the present invention;

[0040] Fig. 6(b) is a schematic view illustrating the subtype of human papilloma viruses identified by each dot shown in Fig. 6(a);

[0041] Fig. 7(a) is a view showing the detector stained with SYBR Green II according to a embodiment of the present invention; and

[0042] Fig. 7(b) is a view showing the detecting result on the detectors of detecting samples according to a preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0043] The present invention will now described more specifically with reference to the following embodiments. Papilloma viruses are small (50-60 nm), nonenveloped, and icosahedral DNA viruses. The DNA of many papilloma viruses, including over 50 human viruses, has been cloned and sequenced. Although there is a high degree of sequence divergence between species, all papilloma viruses share some common features of genome organization. The open reading frames (ORFs) of the virus genomes are designated an early region, a late region, and a long control region (LCR) of transcription. The early region contains genes E1-E8 (not all are present in all species), the late region contains genes L1 and L2 (where "E" denotes early and "L" denotes late), and the long control region (LCR) of transcription includes the promoter and enhancer for the viral early genes and the origin of replication. The early region encodes genes required for viral DNA replication, cellular proliferation, and, in some viruses, cellular transformation. The late region (about 3 kb) codes for the capsid proteins. L1 is the major capsid protein and

is relatively well conserved among all the papilloma virus types. The L1 protein is about 500 amino acids in size. L1 probably induces the major humoral and cell-mediated responses to viral infection. The L2 proteins are about 500 amino acids in size, account for only a small proportion of the virion mass, and their function is not yet clear. The LCR region contains an origin of replication with binding sites for E1 and E2 and other *cis* acting sequences in the promoter and enhancer region.

[0044] Generally, PCR has been considered to be the most sensitive method for identifying HPV subtypes in biological samples. A number of different primer combinations amplifying DNA fragment from various regions of the HPV genome have been developed and used for the detection of HPV. However, primers amplifying DNA fragments in the conserved L1 region have become the most widely used in the clinical and epidemiological studies. It is because that certain region of the L1 gene presents a high degree of sequence variability in different HPV subtypes. In other words, the sequence variability among each HPV subtype could be the specific site for identifying each different HPV subtype.

[0045] In order to identify the various HPV subtypes, the Applicant focuses on the loci near the end of L1 gene to search the specific sequence variability as mentioned above. More specifically, the PCR fragment synthesized by the primer sets MY11/MY09 (as disclosed in Weimin et al., 1997, J. Clin. Microbiol. 35(6): 1304-1310) in the L1 region is the particular loci ranges where the Applicant refers to find the specific sequence variability for each HPV subtype in the present invention. Since the specific sequence variability for each HPV subtype is not only specific to a particular HPV subtype, but also distinguished from any other HPV subtype, consequently, the

probes specifically hybridization with a particular HPV subtype could be selected for identifying or diagnosing HPV subtypes, which is also one of the main purposes of the present invention.

[0046] The PCR fragments synthesized by the primer sets MY11/MY09 in the L1 region are about 450 bp in length and had been published. The sequences of the fragments for each HPV subtype described in the invention are publicly available, for example, from the National Center for Biotechnology Information (NCBI) (e.g., www.ncbi.nih.gov). The 39 HPV subtypes identified in the invention includes HPV 6, HPV 11, HPV 16, HPV 18, HPV 26, HPV 31, HPV 32, HPV 33, HPV 35, HPV 37, HPV 39, HPV 42, HPV 43, HPV 44, HPV 45, HPV 51, HPV 52, HPV 53, HPV 54, HPV 55, HPV 56, HPV 58, HPV 59, HPV 61, HPV 62, HPV 66, HPV 67, HPV 68, HPV 69, HPV 70, HPV 72, HPV 74, HPV 82, HPV CP8061, HPV CP8034, HPV L1AE5, HPV MM4, HPV MM7 and HPV MM8. The original NCBI Accession number and the loci of the PCR fragments synthesized by the primer sets MY11/MY09 for different HPV subtypes are listed in Table 1:

Table 1

HPV subtype	Accession number/length(bp)	loci / length(bp)	SEQ ID NO.
HPV 6	NC_000904/8012	6743 – 7151/409	430
HPV 11	NC_001525/7931	6727 – 7135/409	431
HPV 16	NC_001526/7904	6602 – 7013/412	432
HPV 18	NC_001357/7857	6578 – 6992/415	433
HPV 26	NC_001583/7855	6553 – 6967/415	434
HPV 31	NC_001527/7912	6520 – 6931/412	435
HPV 32	NC_001586/7961	6837 – 7245/409	436
HPV 33	NC_001528/7909	6559 – 6967/409	437
HPV 35	NC_001529/7851	6542 – 6953/412	438
HPV 37	NC_001687/7421	6711 – 7125/415	439

HPV 39	NC_001535/7833	6605 – 7019/415	440
HPV 42	NC_001534/7917	6802–7210/409	441
HPV 43	U12504/455	21–435/415	442
HPV 44	NC_001689/7833	6647 – 7061/415	443
HPV 45	NC_001590/7858	6582 – 6996/415	444
HPV 51	NC_001533/7808	6486 – 6897/412	445
HPV 52	NC_001592/7942	6623 – 7031/409	446
HPV 53	NC_001593/7856	6614 – 7022/409	447
HPV 54	NC_001676/7759	6561 – 6972/412	448
HPV 55	NC_001692/7822	6647-7061/415	449
HPV 56	NC_001594/7844	6559 – 6967/409	450
HPV 58	NC_001443/7824	6608 – 7016/409	451
HPV 59	NC_001635/7896	6571 – 6985/415	452
HPV 61	NC_001694/7989	6732 – 7146/415	453
HPV 62	U12499/449	21 – 429/409	454
HPV 66	NC_001695/7824	6609 – 7017/409	455
HPV 67	D21208/7801	6584 – 6992/409	456
HPV 68	M73258/6042	2582 – 2996/415	457
HPV 69	NC 002171/7700	6509 – 6923/415	458
HPV 70	NC 001711/7905	6549 – 6963/415	459
HPV 72	X94164/7988	6758 – 7172/415	460
HPV 74	U40822/3891	1613 – 2027/415	461
HPV 82	AB027021/7871	6536 – 6950/415	462
HPV CP8061	U12479/452	21 – 432/412	463
HPV CP8304	U12480/452	21 – 432/412	464
HPV L1AE5	AF039910/364	11 – 360/350	465
HPV MM4	U12488/455	21 – 435/415	466
HPV MM7	U12489/452	21 – 432/412	467
HPV MM8	U12490/452	21 – 432/412	468

[0047] The sequences of the fragments of each HPV subtype described in the invention are listed below:

[0048] Human Papilloma Virus subtype 6 (6743-7151/409 bp)

SEQ ID NO 430

tatttgttgg ggtaatcaac tgtttgttac tgtggtagat accacacgca gtaccaacat 60

gacattatgt gcatccgtaa ctacatcttc cacatacacc aattctgatt ataaagagta 120
catgcgtcat gtggaagagt atgatttaca atttattttt caattatgta gcattacatt 180
gtctgctgaa gtaatggcct atattcacac aatgaatccc tctgttttgg aagactggaa 240
ctttgggtta tcgcctcccc caaatggtac attagaagat acctataggt atgtgcagtc 300
acaggccatt acctgtcaaa agcccactcc tgaaaaggaa aagccagatc cctataagaa 360
ccttagtttt tgggaggtta atttaaaaga aaagttttct agtgaattg 409

[0049] Human Papilloma Virus subtype 11 (6727-7135/409 bp)

SEQ ID NO 431

tatttgctgg ggaaaccact tgtttgttac tgtggtagat accacacgca gtacaaatat 60
gacactatgt gcatctgtgt ctaaatctgc tacatacact aattcagatt ataaggaata 120
catgcgccat gtggaggagt ttgatttaca gtttattttt caattgtgta gcattacatt 180
atctgcagaa gtcatggcct atatacacac aatgaatcct tctgttttgg aggactggaa 240
ctttggttta tcgcctccac caaatggtac actggaggat acttatagat atgtacagtc 300
acaggccatt acctgtcaga aacccacacc tgaaaaagaa aaacaggatc cctataagga 360
tatgagtttt tgggaggtta acttaaaaga aaagttttca agtgaatta 409

[0050] Human Papilloma Virus subtype 16 (6602-7013/412 bp)

SEQ ID NO 432

catttgttgg ggtaaccaac tatttgttac tgttgttgat actacacgca gtacaaatat 60
gtcattatgt gctgccatat ctacttcaga aactacatat aaaaatacta actttaagga 120
gtacctacga catggggagg aatatgattt acagtttatt tttcaactgt gcaaaataac 180
cttaactgca gacgttatga catacataca ttctatgaat tccactattt tggaggactg 240
gaattttggt ctacaacctc ccccaggagg cacactagaa gatacttata ggtttgtaac 300
ccaggcaatt gcttgtcaaa aacatacacc tccagcacct aaagaagatg atccccttaa 360
aaaatacact ttttgggaag taaatttaaa ggaaaagttt tctgcagacc ta 412

[0051] Human Papilloma Virus subtype 18 (6587-6992/415 bp)

SEQ ID NO 433

tgtttgctgg cataatcaat tatttgttac tgtggtagat accactccca gtaccaattt 60
aacaatatgt gcttctacac agtctcctgt acctgggcaa tatgatgcta ccaaatttaa 120
gcagtatagc agacatgttg aggaatatga tttgcagttt atttttcagt tgtgtactat 180
tactttaact gcagatgtta tgtcctatat tcatagtatg aatagcagta ttttagagga 240
ttggaacttt ggtgttcccc ccccccccaac tactagtttg gtggatacat atcgttttgt 300
acaatctgtt gctattacct gtcaaaagga tgctgcaccg gctgaaaata aggatcccta 360

tgataagtta aagttttgga atgtggattt aaaggaaaag ttttctttag actta 415

[0052] Human Papilloma Virus subtype 26 (6553-6967/415 bp)

SEQ ID NO 434

tatctgttgg ggcaatcaat tgtttgttac ctgtgttgat accacccgca gtactaacct 60
taccattagt acattatctg cagcatctgc atccactcca tttaaaccat ctgattataa 120
acaatttata agacatggcg aagaatatga attacaattt atatttcagt tgtgtaaaat 180
aacacttaca acagatgtta tggcttacat acatttaatg aatgcctcca tattggagga 240
ttggaatttt ggactaacct tacctcccac tgctagtttg gaagatgcct ataggtttat 300
taaaaactct gctactacct gtcagcgtaa cgcccctcct gtgccaaagg aagatccttt 360
tcaaaaattt aaattttggg atgtagattt aaaagaaaaa ttttctattg atttg 415

[0053] Human Papilloma Virus subtype 31 (6520-6931/412 bp)

SEQ ID NO 435

tatttgttgg ggcaatcagt tatttgttac tgtggtagat accacacgta gtaccaatat 60
gtctgtttgt gctgcaattg caaacagtga tactacattt aaaagtagta attttaaaga 120
gtatttaaga catggtgagg aatttgattt acaatttata tttcagttat gcaaaataac 180
attatctgca gacataatga catatattca cagtatgaat cctgctattt tggaagattg 240
gaattttgga ttgaccacac ctccctcagg ttctttggag gatacctata ggtttgtcac 300
ctcacaggcc attacatgtc aaaaaactgc cccccaaaag cccaaggaag atccatttaa 360
agattatgta ttttgggagg ttaatttaaa agaaaagttt tctgcagatt ta 412

[0054] Human Papilloma Virus subtype 32 (6837-7245/409 bp)

SEQ ID NO 436

tatatgttgg ggtaatcaag tgtttctaac tgttgtggat actacccgta gtactaacat 60
gactgtgtgt gctactgtaa caactgaaga cacatacaag tctactaact ttaaggaata 120
tctacgccat gcagaggaat atgatataca gtttatattt caattgtgca aaattacatt 180
atctgtagag gttatgtcat atatccacac catgaatcct gacatactag acgattggaa 240
tgttggtgta gctccaccgc cctctggtac tttagaagat agttatagat ttgtgcagtc 300
tcaggccata cgatgtcaag ctaaggtaac agcacctgaa aaaaaggatc cttttctga 360
ctattcattt tgggaagtaa atttatctga aaagttttct agtgattta 409

[0055] Human Papilloma Virus subtype 33 (6559-6967/409 bp)

SEQ ID NO 437

tatttgttgg ggcaatcagg tatttgttac tgtggtagat accactcgca gtactaatat 60
gactttatgc acacaagtaa ctagtgacag tacatataaa aatgaaaatt ttaaagaata 120
tataagacat gttgaagaat atgatctaca gtttgttttt caactatgca aagttacctt 180
aactgcagaa gttatgacat atattcatgc tatgaatcca gatattttag aagattggca 240
atttggttta acacctcctc catctgctag tttacaggat acctataggt ttgttacctc 300
tcaggctatt acgtgtcaaa aaacagtacc tccaaaggaa aaggaagacc ccttaggtaa 360
atatacattt tgggaagtgg atttaaagga aaaattttca gcagattta 409

[0056] Human Papilloma Virus subtype 35 (6542-6953/412 bp)

SEQ ID NO 438

tatttgttgg agtaaccaat tgtttgttac tgtagttgat acaacccgta gtacaaatat 60
gtctgtgtgt tctgctgtgt cttctagtga cagtacatat aaaaatgaca attttaagga 120
atatttaagg catggtgaag aatatgattt acagtttatt tttcagttat gtaaaataac 180
actaacagca gatgttatga catatattca tagtatgaac ccgtccattt tagaggattg 240
gaattttggc cttacaccac cgccttctgg taccttagag gacacatatc gctatgtaac 300
atcacaggct gtaacttgtc aaaaacccag tgcaccaaaa cctaaagatg atccattaaa 360
aaattatact ttttgggagg ttgatttaaa ggaaaagttt tctgcagact ta 412

[0057] Human Papilloma Virus subtype 37 (6711-7125/415 bp)

SEQ ID NO 439

cattttatgg ggtaatcaaa tgtttatcac agttgctgat aatacacgga acacaaactt 60
ttctattagt gtgtctactg acaatggcga agttacagaa tataattctc aaacactcag 120
agaataccta agacatgttg aagaatacca gctttcaatt attttacaac tttgtaaagt 180
tcctttaaag gctgaggttt taactcagat aaatgcaatg aattctggta tattggaaga 240
gtggcaatta ggatttgtac ctactccaga taattcagta catgaccttt ataggtacat 300
taattcaaag gctaccaagt gtcctgatgc agttgttgaa aaagaaaagg aagatccctt 360
tgcaaaatat acattttgga atgtagattt aactgaaaaa ttatcattgg attta 415

[0058] Human Papilloma Virus subtype 39 (6605-7017/415 bp)

SEQ ID NO 440

tatatgttgg cataatcaat tatttcttac tgttgtggac actacccgta gtaccaactt 60
tacattatct acctctatag agtcttccat accttctaca tatgatcctt ctaagtttaa 120
ggaatatacc aggcacgtgg aggagtatga tttacaattt atatttcaac tgtgtactgt 180
cacattaaca actgatgtta tgtcttatat tcacactatg aattcctcta tattggacaa 240

ttggaatttt gctgtagctc ctccaccatc tgccagtttg gtagacactt acagatacct 300
acagtctgca gccattacat gtcaaaagga tgctccagca cctgaaaaga aagatccata 360
tgacggtcta aagttttgga atgttgactt aagggaaaag tttagtttgg aactt 415

[0059] Human Papilloma Virus subtype 42 (6802-7210/409 bp)

SEQ ID NO 441

tatatgttgg ggaaatcagc tatttttaac tgtggttgat actacccgta gtactaacat 60
gactttgtgt gccactgcaa catctggtga tacatataca gctgctaatt ttaaggaata 120
tttaagacat gctgaagaat atgatgtgca atttatattt caattgtgta aaataacatt 180
aactgttgaa gttatgtcat atatacacaa tatgaatcct aacatattag aggagtggaa 240
tgttggtgtt gcaccaccac cttcaggaac tttagaagat agttataggt atgtacaatc 300
agaagctatt cgctgtcagg ctaaggtaac aacgccagaa aaaaaggatc cttattcaga 360
cttttggttt tgggaggtaa atttatctga aaagttttct actgattta 409

[0060] Human Papilloma Virus subtype 43 (21-435/415 bp)

SEQ ID NO 442

catttgtttt gggaatcagt tgtttgttac agtggtagat accactcgta gtacaaactt 60
gacgttatgt gcctctactg accctactgt gcccagtaca tatgacaatg caaagtttaa 120
ggaatacttg cggcatgtgg aagaatatga tctgcagttt atatttcaat tatgcataat 180
aacgctaaac ccagaggtta tgacatatat tcatactatg gatcccacat tattagagga 240
ctggaatttt ggtgtgtccc cacctgcctc tgcttctttg gaagatactt atcgcttttt 300
gtctaacaag gccattgcat gtcaaaaaaa tgctccccca aaggaacggg aggatcccta 360
taaaaagtat acattttggg atataaatct tacagaaaag ttttctgcac aactt 415

[0061] Human Papilloma Virus subtype 44 (6647-7061/415 bp)

SEQ ID NO 443

tatttgttgg ggaaatcagt tatttgttac tgttgtagat actacccgta gtacaaacat 60
gacaatatgt gctgccacta cacagtcccc tccgtctaca tatactagtg aacaatataa 120
gcaatacatg cgacatgttg aggagtttga cttacaattt atgtttcaat tatgtagtat 180
taccttaacg gcggaggtaa tggcctatct tcatactatg aatgctggta ttttagaaca 240
gtggaacttt gggttgtcgc cgcccccaaa tggtacctta gaggacaaat acagatatgt 300
gcagtcccag gccattacat gtcaaaagcc acccccctgaa aaggcaaagc aggaccccta 360
tgcaaaatta agtttttggg aggtggatct tagagaaaag ttttctagtg agttg 415

[0062] Human Papilloma Virus subtype 45 (6582-6996/415 bp)

SEQ ID NO 444

```
tatttgttgg cataatcagt tgtttgttac tgtagtggac actacccgca gtactaattt   60
aacattatgt gcctctacac aaaatcctgt gccaagtaca tatgacccta ctaagtttaa  120
gcagtatagt agacatgtgg aggaatatga tttacagttt atttttcagt tgtgcactat  180
tactttaact gcagaggtta tgtcatatat ccatagtatg aatagtagta tattagaaaa  240
ttggaatttt ggtgtccctc caccacctac tacaagtttg gtggatacat atcgttttgt  300
gcaatcagtt gctgttacct gtcaaaagga tactacacct ccagaaaagc aggatccata  360
tgataaatta aagttttgga ctgttgacct aaaggaaaaa ttttcctccg atttg        415
```

[0063] Human Papilloma Virus subtype 51 (6486-6897/412 bp)

SEQ ID NO 445

```
catttgctgg aacaatcagc tttttattac ctgtgttgat actaccagaa gtacaaattt   60
aactattagc actgccactg ctgcggtttc cccaacattt actccaagta actttaagca  120
atatattagg catggggaag agtatgaatt gcaatttatt tttcaattat gtaaaattac  180
tttaactaca gaggtaatgg cttatttaca cacaatggat cctaccattc ttgaacagtg  240
gaattttgga ttaacattac ctccgtctgc tagtttggag gatgcatata ggtttgttag  300
aaatgcagct actagctgtc aaaaggacac ccctccacag gctaagccag atcctttggc  360
caaatataaa ttttgggatg ttgatttaaa ggaacgattt tctttagatt ta           412
```

[0064] Human Papilloma Virus subtype 52 (6623-7031/409 bp)

SEQ ID NO 446

```
catatgttgg ggcaatcagt tgtttgtcac agttgtggat accactcgta gcactaacat   60
gactttatgt gctgaggtta aaaaggaaag cacatataaa aatgaaaatt ttaaggaata  120
ccttcgtcat ggcgaggaat ttgatttaca atttatttt caattgtgca aaattacatt  180
aacagctgat gttatgacat acattcataa gatggatgcc actattttag aggactggca  240
atttggcctt accccaccac cgtctgcatc tttggaggac acatacagat ttgtcacttc  300
tactgctata acttgtcaaa aaaacacacc acctaaagga aaggaagatc ctttaaagga  360
ctatatgttt tgggaggtgg atttaaaaga aaagttttct gcagattta              409
```

[0065] Human Papilloma Virus subtype 53 (6614-7022/409 bp)

SEQ ID NO 447

catctgttgg aacaatcagt tatttgtaac tgttgtggat accaccagga atacaaacat 60
gactctttcc gcaaccacac agtctatgtc tacatataat tcaaagcaaa ttaaacagta 120
tgttagacat gcagaggaat atgaattaca atttgtgttt caactatgta aaatatccct 180
gtctgctgag gttatggcct atttacatac tatgaattct accttactgg aagactggaa 240
tataggtttg tcgcctcctg ttgccactag cttagaggac aaatacagat atgtgaaaag 300
tgcagctata acctgtcaaa aggatcagcc ccctcctgaa aagcaggacc cactatctaa 360
atataaattt tgggaggtca atttgcaaaa cagtttttct gctgatttg 409

[0066] Human Papilloma Virus subtype 54 (6561-6972/412 bp)

SEQ ID NO 448

tatttgttgg ggcaatcagg tgtttttaac agttgtagat accacccgta gtactaacct 60
aacattgtgt gctacagcat ccacgcagga tagctttaat aattctgact ttagggagta 120
tattagacat gtggaggaat atgatttaca gtttatattt cagttatgta ccataaccct 180
tacagcagat gttatggcct atattcatgg aatgaatccc actattctag aggactggaa 240
ctttggtata acccccccag ctacaagtag tttggaggac acatataggt ttgtacagtc 300
acaggccatt gcatgtcaaa agaataatgc ccctgcaaag gaaaaggagg atccttacag 360
taaatttaat ttttggactg ttgaccttaa ggaacgattt tcatctgacc tt 412

[0067] Human Papilloma Virus subtype 55 (6647-7061/415 bp)

SEQ ID NO 449

tatttgttgg gggaatcagt tatttgttac tgttgtagat actacacgta gtacaaacat 60
gacaatatgt gctgctacaa ctcagtctcc atctacaaca tataatagta cagaatataa 120
acaatacatg cgacatgttg aggagtttga cttacagttt atgtttcaat tatgtagtat 180
taccttaact gctgaggtaa tggcctattt acataccatg aatcctggta ttttggaaca 240
gtggaacttt gggttgtcgc cacccccaaa tggtacctta gaagacaaat acagatatgt 300
gcagtcacag gccattacat gtcaaaagcc tccccctgaa aaggcaaagc aggaccccta 360
tgcaaaatta agtttttggg aggtagatct cagagaaaag ttttctagtg agtta 415

[0068] Human Papilloma Virus subtype 56 (6559-6967/409 bp)

SEQ ID NO 450

catttgctgg ggtaatcaat tatttgttac tgtagtagat actactagaa gtactaacat 60
gactattagt actgctacag aacagttaag taaatatgat gcacgaaaaa ttaatcagta 120
ccttagacat gtggaggaat atgaattaca atttgttttt caattatgca aaattacttt 180
gtctgcagag gttatggcat atttacataa tatgaatgct aacctactgg aggactggaa 240

tattgggtta tccccgccag tggccaccag cctagaagat aaatatagat atgttagaag 300
cacagctata acatgtcaac gggaacagcc accaacagaa aaacaggacc cattagctaa 360
atataaattt tgggatgtta acttacagga cagtttttct acagacctg 419

[0069] Human Papilloma Virus subtype 58 (6608-7016/409 bp)

SEQ ID NO 451

catttgctgg ggcaatcagt tatttgttac cgtggttgat accactcgta gcactaatat 60
gacattatgc actgaagtaa ctaaggaagg tacatataaa aatgataatt ttaaggaata 120
tgtacgtcat gttgaagaat atgacttaca gtttgttttt cagctttgca aaattacact 180
aactgcagag ataatgacat atatacatac tatggattcc aatattttgg aggactggca 240
atttggttta acacctcctc cgtctgccag tttacaggac acatatagat ttgttacctc 300
ccaggctatt acttgccaaa aaacagcacc ccctaaagaa aaggaagatc cattaaataa 360
atatactttt tgggaggtta acttaaagga aaagttttct gcagatcta 409

[0070] Human Papilloma Virus subtype 59 (6571-6985/415 bp)

SEQ ID NO 452

tatatgttgg cacaatcaat tgtttttaac agttgtagat actactcgca gcaccaatct 60
ttctgtgtgt gcttctacta cttcttctat tcctaatgta tacacaccta ccagttttaa 120
agaatatgcc agacatgtgg aggaatttga tttgcagttt atatttcaac tgtgtaaaat 180
aacattaact acagaggtaa tgtcatacat tcataatatg aataccacta ttttggagga 240
ttggaatttt ggtgttacac cacctcctac tgctagttta gttgacacat accgttttgt 300
tcaatctgct gctgtaactt gtcaaaagga caccgcaccg ccagttaaac aggaccctta 360
tgacaaacta aagttttggc ctgtagatct taaggaaagg ttttctgcag atctt 415

[0071] Human Papilloma Virus subtype 61 (6732-7146/415 bp)

SEQ ID NO 453

tatttgttgg tttaatgaat tgtttgtaac cgttgtggat accacccgca gtactaattt 60
aaccatttgt actgctacat ccccccctgt atctgaatat aaagccacaa gctttaggga 120
atatttgcgc catacagagg agtttgattt gcaatttatt tttcagttat gtaaaataca 180
tttaacccct gaaattatgg cctacctaca taatatgaat aaggccttgt tggatgactg 240
gaactttggt gtggtaccac caccctctac cagtttagaa gacacatata ggtttttgca 300
gtccagagct attacatgtc agaagggtgc tgctgccccg ccgcccaagg aggatcgcta 360
tgccaagtta tccttttgga ctgttgattt acgagacaag ttttccactg atttg 415

[0072] Human Papilloma Virus subtype 62 (21-429/409 bp)

SEQ ID NO 454

```
tatttgttgg tttaatgaac tgtttgttac tgtggtggat actaccagaa gtactaattt   60
tactatttgt accgcctcca ctgctgcagc agaatacacg gctaccaact ttagggaatt  120
tttgcgacac acggaggaat ttgatttgca atttatattt caattgtgca aaatacagtt  180
aacccccgaa attatggcct acctgcataa tatgaacaag gacctttgg atgactggaa   240
ctttggggtt ttacctcccc cttccactag tttagatgag acatatcact atttcgagtc  300
tcgggctatt acatgtcaaa gggggctgcc tacccgtccc aaggtggacc cgtatgcgca  360
aatgacattt tggactgtgg atcttaagga caagttgtct actgatttg              409
```

[0073] Human Papilloma Virus subtype 66 (6609-7017/409 bp)

SEQ ID NO 455

```
catatgctgg ggtaatcagg tatttgttac tgttgtggat actaccagaa gcaccaacat   60
gactattaat gcagctaaaa gcacattaac taaatatgat gcccgtgaaa tcaatcaata  120
ccttcgccat gtggaggaat atgaactaca gtttgtgttt caactttgta aaataacctt  180
aactgcagaa gttatggcat atttgcataa tatgaataat actttattag acgattggaa  240
tattggctta tccccaccag ttgcaactag cttagaggat aaatataggt atattaaaag  300
cacagctatt acatgtcaga gggaacagcc ccctgcagaa aagcaggatc ccctggctaa  360
atataagttt tgggaagtta atttacagga cagcttttct gcagacctg              409
```

[0074] Human Papilloma Virus subtype 67 (6584-6992/409 bp)

SEQ ID NO 456

```
tatatgctgg ggtaatcaaa tatttgttac tgttgtagac actacacgta gtaccaacat   60
gactttatgt tctgaggaaa aatcagaggc tacatacaaa aatgaaaact ttaaggaata  120
ccttagacat gtggaagaat atgatttgca gtttatattt cagctgtgca aaatatccct  180
tactgcaaat gttatgcaat acatacacac catgaatcca gatatattag aggactggca  240
atttggcctt acaccacctc cttcaggtaa tttacaggac acatatagat ttgttacctc  300
gcaggctatt acctgtcaaa aaacatcccc tccaacagca aaggaagatc ctcttaaaaa  360
gtacagtttt tgggaaatca atttaaagga aaaattttct gcagattta               409
```

[0075] Human Papilloma Virus subtype 68 (2582-2996/415 bp)

SEQ ID NO 457

```
tatttgttgg cataatcaat tatttcttac tgttgtggat accactcgca gtaccaattt   60
```

tactttgtct actactactg aatcagctgt accaaatatt tatgatccta ataaatttaa 120
ggaatatatt aggcatgttg aggaatatga tttgcaattt atatttcagt tgtgtactat 180
aacattgtcc actgatgtaa tgtcctatat acatactatg aatcctgcta ttttggatga 240
ttggaatttt ggtgttgccc ctccaccatc tgctagtctt gtagatacat accgctatct 300
gcaatcagca gcaattacat gtcaaaaaga cgcccctgca cctactaaaa aggatccata 360
tgatggctta aacttttgga atgtaaattt aaaggaaaag tttagttctg aactg 415

[0076] Human Papilloma Virus subtype 69 (6509-6923/415 bp)

SEQ ID NO 458

catttgttgg ggcaaccaat tgtttgttac ttgtgtagat actacccgca gtaccaacct .. 60
cactattagt actgtatctg cacaatctgc atctgccact tttaaaccat cagattataa 120
gcagtttata aggcatggtg aggaatatga attacagttt atatttcaat tgtgtaaaat 180
tactcttacc actgatgtaa tggcctatat ccatacaatg aattctacta ttttggaaaa 240
ttggaatttt ggccttacct tgcctcctac tgctagtttg gaagatgcat ataggtttat 300
taaaaattca gctactacat gtcaacgcga tgcccctgca cagcccaagg aggatccatt 360
tagtaaatta aaattttggg acgttgatct taaagaaaag ttttctattg attta 415

[0077] Human Papilloma Virus subtype 70 (6549-6963/415 bp)

SEQ ID NO 459

catttgttgg cataaccagt tgtttattac tgtggtggac actacacgta gtactaattt 60
tacattgtct gcctgcaccg aaacggccat acctgctgta tatagcccta caaagtttaa 120
ggaatatact aggcatgtgg aggaatatga tttacaattt atatttcaat tgtgtactat 180
cacattaact gctgacgtta tggcctacat ccatactatg aatcctgcaa ttttggacaa 240
ttggaatata ggagttaccc ctccaccatc tgcaagcttg gtggacacgt ataggtattt 300
acaatcagca gctatagcat gtcaaaagga tgctcctaca cctgaaaaaa aggatcccta 360
tgacgattta aaattttgga atgttgattt aaaggaaaag tttagtacag aacta 415

[0078] Human Papilloma Virus subtype 72 (6758-7172/415 bp)

SEQ ID NO 460

catctgttgg tttaatgagc tttttgtgac agttgtagat actactcgca gtactaatgt 60
aactatttgt actgccacag cgtcctctgt atcagaatat acagcttcta attttcgtga 120
gtatcttcgc cacactgagg aatttgattt gcagtttata tttcaactgt gtaaaattca 180
cttaactcct gaaattatgg cctacttgca caatatgaat aaggccttat tggatgactg 240
gaattttggt gtggtgcctc ctccttctac cagtttggat gatacctata ggtttttgca 300

gtctcgtgcc attacctgtc aaaaggggggc tgccacccct cctcctaaag aagatccata 360
tgctaactta tccttttgga ctgtggattt aaaggacaaa ttttccactg acttg 415

[0079] Human Papilloma Virus subtype 74 (1613-2027/415 bp)

SEQ ID NO 461

tatttgttgg ggtaatcaat tatttgttac agttgtggat accacacgca gtactaacat 60
gactgtgtgt gctcctacct cacaatcgcc ttctgctaca tataatagtt cagactacaa 120
acaatacatg cgacatgtgg aggaatttga tttgcaattt atttttcaat tatgtagtat 180
taagttaact gctgaggtta tggcctatat tcatactatg aatcctacag ttttagaaga 240
gtggaacttt gggctaacgc ctccccccaa tggtacttta gaagacacct acagatatgt 300
gcagtcccag gctattacat gtcaaaaacc tacgcctgat aaagcaaagc ccaatcccta 360
tgcaaattta agtttttggg aagttaatct taaggaaaag ttttctagtg aatta 415

[0080] Human Papilloma Virus subtype 82 (6536-6950/415 bp)

SEQ ID NO 462

catttgctgg aataatcagc tttttattac ttgtgttgac actactaaaa gtaccaattt 60
aaccattagc actgctgtta ctccatctgt tgcacaaaca tttactccag caaactttaa 120
gcagtacatt aggcatgggg aagaatatga attgcaattt atatttcaat tgtgtaaaat 180
cactttaact actgaaatta tggcttacct gcacaccatg gattctacaa ttttagaaca 240
gtggaatttt ggattaacat tgccccctc cgctagtttg gaggatgcct atcgatttgt 300
aaaaaatgca gcaacatcct gtcacaagga cagtcctcca caggctaaag aagacccttt 360
ggcaaaatat aaattttgga atgtagacct taaggaacgc ttttctttgg atttg 415

[0081] Human Papilloma Virus subtype CP8061 (21-432/412 bp)

SEQ ID NO 463

catttgttgg ggcaatcagc tttttgtaac agttgtggac acatcacgta gtacaaatat 60
gtccatctgt gctaccaaaa ctgttgagtc tacatataaa gcctctagtt tcatggaata 120
tttgagacat ggagaagaat ttgatttgca atttatattt caactatgtg ttattaattt 180
aacagctgaa attatggcct acttacatcg catggatgct acattactgg aggactggaa 240
tttttggttc ttaccacctc ctactgctag tcttggtgat acctaccgct ttttacagtc 300
tcaggccata acctgtcaga aaaacagtcc tcctcctgca gaaaaaaagg acccctatgc 360
agatcttaca ttttgggagg tggatttaaa ggagcggttt tcactagaat tg 412

[0082] Human Papilloma Virus subtype CP8304 (21-432/412 bp)

SEQ ID NO 464

```
tatttgttgg tttaatgaaa tgtttgttac agtggtggat actaccagaa gcaccaattt   60
tactatttgc acagctacat ctgctgctgc agaatacaag gcctctaact ttaaggaatt  120
tctgcgccat acagaggaat atgatttgca gtttattttc caattatgta aaatacagtt  180
aacaccagaa attatggcct acttacataa tatgaacaag gcactgttgg atgattggaa  240
ttttggtgtg ttgccacctc cttccaccag tttagatgac acatatcgct ttttacagtc  300
tcgggccatt acctgtcaaa agggtgctgc tgcccctgcg cccaaagagg acccttatgc  360
cgacatgtca ttttggacag ttgaccttaa ggacaagttg tctactgatt tg           412
```

[0083] Human Papilloma Virus subtype L1AE5 (11-360/350 bp)

SEQ ID NO 465

```
ggcacaacca attatttata actgtggtag acacaacacg tagtaccaat cttaccttat   60
ctactgcaac tactaatcca gttccatcta tatatgaacc ttctaaattt aaggaataca  120
cacgccatgt agaggaatat gatttacaat ttatatttca attgtgtaaa attacactta  180
ctactgatgt tatgtcttat atacataaca tggatcctac tattttagat agttggaatt  240
ttggtgttag tcctccccca tctgctagct tagtagatac atataggttt ttacagtcat  300
ctgccattac atgtcagaag gatgtggttg ttccacaaaa aaaggatcca             350
```

[0084] Human Papilloma Virus subtype MM4 (21-435/415 bp)

SEQ ID NO 466

```
catttgctgg aataatcagc tttttattac ttgtgttgac actactagaa gtaccaattt   60
aaccattagc actgctgtta ctcaatctgt tgcacaaaca tttactccag caaactttaa  120
gcaatacatt aggcatgggg aagaatatga attgcaattt atatttcaat tgtgtaaaat  180
cactttaact actgaaatta tggcttacct gcacaccatg gattctacaa ttttagaaca  240
gtggaatttt ggattaacct tgcccccctc agctagtttg gaggatgcct atcgatttgt  300
aaaaaatgca gcaacatcct gtcacaagga cagtcctcca caggctaaac aagacccttt  360
ggcaaaatat aaattttgga atgtagacct taaggaacgc ttttctttgg atttg       415
```

[0085] Human Papilloma Virus subtype MM7 (21-432/412 bp)

SEQ ID NO 467

```
catttgttgg tttaatgagt tatttgttac agttgtagat actacccgca gtaccaatat   60
tactatttca gctgctgcta cacaggctaa tgaatacaca gcctctaact ttaaggaata  120
cctccgccac accgaggaat atgacttaca ggttatattg caactttgca aaatacatct  180
```

```
taccctgaa attatggcat acctacatag tatgaatgaa catttattgg atgagtggaa   240
ttttggcgtg ttaccacctc cttccaccag ccttgatgat acctatcgct atctgcagtc   300
ccgtgctatt acctgccaaa agggtccttc cgcccctgcc cctaaaaagg atccttatga   360
tggccttgta ttttgggagg ttgatttaaa ggacaaacta tccacagatt tg           412
```

[0086] Human Papilloma Virus subtype MM8 (21-432/412 bp)

SEQ ID NO 468

```
tatatgctgg tttaatcaat tgtttgtcac ggtggtggat accacccgca gcaccaattt    60
tactattagt gctgctacca acaccgaatc agaatataaa cctaccaatt ttaaggaata   120
cctaagacat gtggaggaat atgatttgca gtttatattc cagttgtgta aggtccgtct   180
gactccagag gtcatgtcct atttacatac tatgaatgac tccttattag atgagtggaa   240
ttttggtgtt gtgccccctc cctccacaag tttagatgat acctataggt acttgcagtc   300
tcgcgccatt acttgccaaa agggggccgc cgccgccaag cctaaggaag atccttatgc   360
tggcatgtcc ttttgggatg tagatttaaa ggacaagttt tctactgatt tg           412
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[0087] In order to find the specific probes for identifying or diagnosing HPV subtypes, some sequence analysis software are used for finding the variety sites among the above listed sequences of different HPV subtypes, e.g., DNASTAR. The above 450-bp sequences of 39 HPV subtypes are respectively divided into several fragments and analyzed by the software. Preferably, the genetic identify compared to other HPV subtypes must be lower than 30% for finding suitable probes with high specificity. After identifying the variety sites having low genetic identity in sequences of each HPV subtype, the probes for each HPV subtype are respectively designed to specifically hybridize with these variety sites. Then, the designed probes are tested for their specificities to the corresponding HPV subtypes respectively. Preferably, the probes are 15-30 base pairs in length. Ultimately, 9-12 probes with high specificity are found for each HPV subtype. The sequences of the probes for each HPV subtype are listed below.

HPV 6

SEQ ID NO	5'→ 3'	Locus in HPV 6
1	CATCCGTAACTACATCTTCC	6814 – 6833
2	ATCCGTAACTACATCTTCCA	6815 – 6834
3	CTACATCTTCCACATACACCAA	6823 – 6844
4	CATCTTCCACATACACCAAT	6826 – 6845
5	ATCTTCCACATACACCAATT	6827 – 6846
6	CCACATACACCAATTCTGAT	6832 – 6851
7	TAGCATTACATTGTCTGCTGAAG	6911 – 6933
8	TCCCTCTGTTTTGGAAGAC	6959 – 6977
09	GTTATCGCCTCCCCCAAATGGTACAT	6989 – 7014
10	CTATAGGTATGTGCAGTCACAG	7025 – 7046
11	GCCCACTCCTGAAAAGGAA	7064 – 7082
12	CTATAAGAACCTTAGT	7094 – 7109

HPV 11

SEQ ID NO	5'→ 3'	Locus in HPV 11
13	ATCTGTGTCTAAATC	6799 –6813
14	TCTGTGTCTAAATCTGCTAC	6800 – 6819
15	ATCTGTGTCTAAATCTGCTACATACA	6799 – 6824
16	TGCATCTGTGTCTAAATCTG	6796 – 6815
17	AAATCTGCTACATACACTAA	6809 – 6828
18	CTAAATCTGCTACATACACTA	6807 – 6827
19	CTACATACACTAATTCAGAT	6816 – 6835
20	TAGCATTACATTATCTGCAGAAG	6895 – 6917
21	TCCTTCTGTTTTGGAGGAC	6943 – 6961
22	TTTATCGCCTCCACCAAATGGTACAC	6973 – 6998
23	TTATAGATATGTACAGTCACAGGCC	7009 – 7033
24	ACCCACACCTGAAAAAGAAAAAC	7048 – 7070

HPV 16

SEQ ID NO	5'→ 3'	Locus in HPV 16
25	TATGTCATTATGTGCTGCCA	6659 – 6678
26	GTGCTGCCATATCTACTTCA	6670 – 6689
27	TGCCATATCTACTTC	6674 – 6688

28	TATCTACTTCAGAAACTACA	6679 – 6698
29	CTACTTCAGAAACTACATATAA	6682 – 6703
30	ATAAAAATACTAACTTTAAG	6700 – 6719
31	CAAAATAACCTTAACTGCAGACG	6773 – 6795
32	TTCCACTATTTTGGAGGAC	6821 – 6839
33	TCTACAACCTCCCCCAGGAGGCACAC	6851 – 6876
34	TTATAGGTTTGTAACCCAG	6887 – 6905
35	ACATACACCTCCAGCACCT	6923 – 6941
36	CCTTAAAAAATACACT	6956 – 6971

HPV 18

SEQ ID NO	5'→ 3'	Locus in HPV 18
37	TTCTACACAGTCTCC	6650 – 6664
38	CAGTCTCCTGTACCTGGGCA	6657 – 6676
39	AGTCTCCTGTACCTGGGCAA	6658 – 6677
40	TCTCCTGTACCTGGGCAATATGA	6660 – 6682
41	CTGTACCTGGGCAATATGAT	6664 – 6683
42	ATGATGCTACCAAATTTAAG	6679 – 6698
43	TACTATTACTTTAACTGCAGATG	6752 – 6774
44	TAGCAGTATTTTAGAGGAT	6800 – 6818
45	TGTTCCCCCCCCCCCAACTACTAGTT	6830 – 6855
46	ATATCGTTTTGTACAATCTGTT	6866 – 6887
47	GGATGCTGCACCGGCTGAA	6905 – 6923
48	CTATGATAAGTTAAAG	6935 – 6950

HPV 26

SEQ ID NO	5'→ 3'	Locus in HPV 26
49	TAGTACATTATCTGCAGCAT	6619 – 6638
50	ATTATCTGCAGCATC	6625 – 6639
51	TGCAGCATCTGCATCCACTC	6631 – 6650
52	GCATCTGCATCCACTCCATTTAAA	6635 – 6658
53	CTCCATTTAAACCATCTGAT	6648 – 6667
54	TAAAATAACACTTACAACAGATG	6727 – 6749
55	TGCCTCCATATTGGAGGAT	6775 – 6793
56	ACTAACCTTACCTCCCACTGCTAGTT	6805 – 6830
57	CTATAGGTTTATTAAAAACTCT	6841 – 6862
58	TAACGCCCCTCCTGTGCCA	6880 – 6898

HPV 31

SEQ ID NO	5'→ 3'	Locus in HPV 31
59	TGCAATTGCAAACAG	6592 – 6606
60	GCAATTGCAAACAGTGATAC	6593 – 6612
61	CAATTGCAAACAGTGATACT	6594 – 6613
62	GCAAACAGTGATACTACATTTAA	6599 – 6621
63	CTACATTTAAAAGTAGTAAT	6612 – 6631
64	CAAAATAACATTATCTGCAGACA	6691 – 6713
65	TCCTGCTATTTTGGAAGAT	6739 – 6757
66	ATTGACCACACCTCCCTCAGGTTCTT	6769 – 6794
67	CTATAGGTTTGTCACCTCACAG	6805 – 6826
68	AACTGCCCCCCAAAAGCCC	6844 – 6862

HPV 32

SEQ ID NO	5'→ 3'	Locus in HPV 32
69	TGCTACTGTAACAACTGAAG	6906 – 6925
70	GCTACTGTAACAACTGAAGA	6907 – 6926
71	TACTGTAACAACTGA	6909 – 6923
72	ACTGTAACAACTGAAGACAC	6910 – 6929
73	CAACTGAAGACACATACAAGTC	6917 – 6938
74	CAAAATTACATTATCTGTAGAGG	7005 – 7027
75	TCCTGACATACTAGACGAT	7053 – 7071
76	TGTAGCTCCACCGCCCTCTGGTACTT	7083 – 7108
77	TTATAGATTTGTGCAGTCTCAG	7119 –7140
78	TAAGGTAACAGCACCTGAA	7158 – 7176
79	TTTTTCTGACTATTCA	7188 – 7203

HPV 33

SEQ ID NO	5'→ 3'	Locus in HPV 33
80	TATGCACACAAGTAACTAGT	6624 – 6643
81	CACACAAGTAACTAG	6628 – 6642
82	ACAAGTAACTAGTGACAGTA	6631 – 6650
83	GTAACTAGTGACAGTACATATAA	6635 – 6657
84	GTACATATAAAAATGAAAAT	6648 – 6667
85	CAAAGTTACCTTAACTGCAGAAG	6727 – 6749
86	TCCAGATATTTTAGAAGAT	6775 – 6793

87	TTTAACACCTCCTCCATCTGCTAGTT	6805 – 6830
88	CTATAGGTTTGTTACCTCTCAG	6841 – 6862
89	AACAGTACCTCCAAAGGAA	6880 – 6898
90	CTTAGGTAAATATACA	6910 – 6925

HPV 35

SEQ ID NO	5'→ 3'	Locus in HPV 35
91	TCTGCTGTGTCTTCTAGTGA	6612 – 6631
92	TGCTGTGTCTTCTAG	6614 – 6628
93	GTGTCTTCTAGTGACAGTAC	6618 – 6637
94	CTTCTAGTGACAGTACATATAAA	6622 – 6644
95	GTACATATAAAAATGACAAT	6634 – 6653
96	TAAAATAACACTAACAGCAGATG	6713 – 6735
97	CCCGTCCATTTTAGAGGAT	6761 – 6779
98	CCTTACACCACCGCCTTCTGGTACCT	6791 – 6816
99	ATATCGCTATGTAACATCACAG	6827 – 6848
100	ACCCAGTGCACCAAAACCT	6866 – 6884

HPV 37

SEQ ID NO	5'→ 3'	Locus in HPV 37
101	TGTCTACTGACAATG	6782 – 6796
102	TGTCTACTGACAATGGCGAA	6782 – 6801
103	TGACAATGGCGAAGTTACAG	6789 – 6808
104	GACAATGGCGAAGTTACAGA	6790 – 6809
105	AATGGCGAAGTTACAGAATA	6793 – 6812
106	CAGAATATAATTCTCAAACA	6806 – 6825
107	TAAAGTTCCTTTAAAGGCTGAGG	6885 – 6907
108	TTCTGGTATATTGGAAGAG	6933 – 6951
109	ATTTGTACCTACTCCAGATAATTCAG	6963 – 6988
110	TTATAGGTACATTAATTCAAAG	6999 – 7020
111	TGCAGTTGTTGAAAAAGAA	7038 – 7056
112	CTTTGCAAAATATACA	7068 – 7083

HPV 39

SEQ ID NO	5'→ 3'	Locus in HPV 39
113	CTCTATAGAGTCTTC	6677 – 6691
114	TAGAGTCTTCCATACCTTCT	6682 – 6701

115	ATAGAGTCTTCCATACCTTC	6681 – 6700
116	GTCTTCCATACCTTCTACATATG	6686 – 6708
117	CTACATATGATCCTTCTAAG	6700 – 6719
118	TACTGTCACATTAACAACTGATG	6779 – 6801
119	TTCCTCTATATTGGACAA	6827 – 6844
120	TGTAGCTCCTCCACCATCTGCCAGTT	6857 – 6882
121	TTACAGATACCTACAGTCTGCA	6893 – 6914
122	GGATGCTCCAGCACCTGAA	6932 – 6950
123	ATATGACGGTCTAAAG	6962 – 6977

HPV 42

SEQ ID NO	5'→ 3'	Locus in HPV 42
124	TATATGTTGGGGAAATCAGCTA	6802 - 6823
125	CACTGCAACATCTGGTGATA	6874 - 6893
126	GCAACATCTGGTGATACATATACAG CTGCT	6878 - 6907
127	CATTAACTGTTGAAGTTATGTCA	6978 - 7000
128	CCTAACATATTAGAGGAGTGGAATG T	7019 - 7044
129	CACCACCACCTTCAGGAACT	7053 - 7072
130	GTTATAGGTATGTACAATCAGAAG	7083 - 7106
131	GCTAAGGTAACAACGCCAGAAAAAA AGGAT	7121 - 7150
132	CAGACTTTTGGTTTTGGGAGGTAA	7158 - 7181
133	GAAAAGTTTTCTACTGATTTA	7190 - 7210

HPV 43

SEQ ID NO	5'→ 3'	Locus in HPV 43
134	CATTTGTTTTGGGAATCAGTTG	21 - 42
135	TGACCCTACTGTGCCCAGTA	99 - 118
136	ACTGTGCCCAGTACATATGACAATGC AAAG	106 - 135
137	GTTTATATTTCAATTATGCATAA	177 - 199
138	CCAGAGGTTATGACATATATT	211 – 231
139	CCCACATTATTAGAGGACTGGAA	244 - 266
140	CCACCTGCCTCTGCTTCTTTG	280 - 300
141	CGCTTTTTGTCTAACAAGGCCATTG	313 – 337

142	CCAAAGGAACGGGAGGATCCCTA	358 - 380
143	CTTACAGAAAAGTTTTCTGCACAAC	409 - 433

HPV 44

SEQ ID NO	5'→ 3'	Locus in HPV 40
144	TGCCACTACACAGTC	6719 – 6733
145	CTACACAGTCCCCTCCGTCT	6724 – 6743
146	TGCCACTACACAGTCCCCTC	6719 – 6738
147	CAGTCCCCTCCGTCTACATATA	6729 – 6750
148	CTACATATACTAGTGAACAA	6742 – 6761
149	TAGTATTACCTTAACGGCGGAGG	6821 – 6843
150	TGCTGGTATTTTAGAACAG	6869 – 6887
151	GTTGTCGCCGCCCCCAAATGGTACC T	6899 – 6924
152	ATACAGATATGTGCAGTCCCAG	6935 – 6956
153	GCCACCCCCTGAAAAGGCA	6974 – 6992
154	CTATGCAAAATTAAGT	7004 – 7019

HPV 45

SEQ ID NO	5'→ 3'	Locus in HPV 45
155	TGCCTCTACACAAAATCCTG	6651 – 6670
156	CTCTACACAAAATCC	6654 – 6668
157	ACAAAATCCTGTGCCAAGTA	6660 – 6679
158	CAAAATCCTGTGCCAAGTAC	6661 – 6680
159	AATCCTGTGCCAAGTACATATG	6664 – 6685
160	GTACATATGACCCTACTAAG	6677 – 6696
161	CACTATTACTTTAACTGCAGAGG	6756 – 6778
162	TAGTAGTATATTAGAAAAT	6804 – 6822
163	TGTCCCTCCACCACCTACTACAAGTT	6834 – 6859
164	ATATCGTTTTGTGCAATCAGTT	6870 – 6891
165	GGATACTACACCTCCAGAA	6909 – 6927

HPV 51

SEQ ID NO	5'→ 3'	Locus in HPV 51
166	CACTGCCACTGCTGCGGTTT	6555 – 6574
167	TGCCACTGCTGCGGT	6558 – 6572
168	CACTGCTGCGGTTTCCCCAA	6561 – 6580

169	CCACTGCTGCGGTTTCCCCA	6560 – 6579
170	CTGCGGTTTCCCCAACATTTAC	6566 – 6587
171	CAACATTTACTCCAAGTAAC	6578 – 6597
172	TAAAATTACTTTAACTACAGAGG	6657 – 6679
173	TCCTACCATTCTTGAACAG	6705 – 6723
174	ATTAACATTACCTCCGTCTGCTAGTT	6735 – 6760
175	ATATAGGTTTGTTAGAAATGCA	6771 – 6792
176	GGACACCCCTCCACAGGCT	6810 – 6828
177	TTTGGCCAAATATAAA	6840 – 6855

HPV 52

SEQ ID NO	5'→ 3'	Locus in HPV 52
178	TGAGGTTAAAAAGGA	6695 – 6709
179	TGAGGTTAAAAAGGAAAGCA	6695 – 6714
180	GAGGTTAAAAAGGAAAGCAC	6696 – 6715
181	TTAAAAAGGAAAGCACATAT	6700 – 6719
182	AAAGGAAAGCACATATAAAAAT	6704 – 6725
183	GCACATATAAAAATGAAAAT	6712 – 6731
184	CAAAATTACATTAACAGCTGATG	6791 – 6813
185	TGCCACTATTTTAGAGGAC	6839 – 6857
186	CCTTACCCCACCACCGTCTGCATCTT	6869 – 6894
187	ATACAGATTTGTCACTTCTACT	6905 – 6926
188	AAACACACCACCTAAAGGA	6944 – 6962
189	TTTAAAGGACTATATG	6974 – 6989

HPV 53

SEQ ID NO	5'→ 3'	Locus in HPV 53
190	TCCGCAACCACACAGTCTAT	6681 – 6700
191	CCGCAACCACACAGT	6682 – 6696
192	CCGCAACCACACAGTCTATG	6682 – 6701
193	CACAGTCTATGTCTACATATAA	6691 – 6712
194	CTACATATAATTCAAAGCAA	6703 – 6722
195	TAAAATATCCCTGTCTGCTGAGG	6782 – 6804
196	TTCTACCTTACTGGAAGAC	6830 – 6848
197	TTTGTCGCCTCCTGTTGCCACTAGCT	6860 – 6885
198	ATACAGATATGTGAAAAGTGCA	6896 – 6917
199	GGATCAGCCCCCTCCTGAA	6935 - 6953

HPV 54

SEQ ID NO	5'→ 3'	Locus in HPV 54
200	TACAGCATCCACGCA	6633 – 6647
201	CAGCATCCACGCAGGATAGC	6635 – 6654
202	ACGCAGGATAGCTTTAATAA	6643 – 6662
203	CACGCAGGATAGCTTTAATA	6642 – 6661
204	ATAGCTTTAATAATTCTGAC	6650 – 6669
205	TACCATAACCCTTACAGCAGATG	6729 – 6751
206	TCCCACTATTCTAGAGGAC	6777 – 6795
207	TATAACCCCCCCAGCTACAAGTAGT T	6807 – 6832
208	ATATAGGTTTGTACAGTCACAG	6843 – 6864
209	GAATAATGCCCCTGCAAAGGAA	6882 – 6903

HPV 55

SEQ ID NO	5'→ 3'	Locus in HPV 55
210	TTTGTTACTGTTGTAGATACTAC	6669 - 6691
211	ATGACAATATGTGCTGCTAC	6705 - 6724
212	GACAATATGTGCTGCTACAA	6707 - 6726
213	TGCTACAACTCAGTCTCCAT	6719 - 6738
214	CTACAACTCAGTCTCCATCT	6721 - 6740
215	ACAACTCAGTCTCCATCTAC	6723 - 6742
216	ATGTTGAGGAGTTTGACTTA	6781 - 6800
217	TGTTGAGGAGTTTGACTTAC	6782 - 6801
218	TGAGGAGTTTGACTTACAGT	6785 - 6804

HPV 56

SEQ ID NO	5'→ 3'	Locus in HPV 56
219	CTGCTACAGAACAGT	6630 – 6644
220	GCTACAGAACAGTTAAGTAA	6632 – 6651
221	CAGAACAGTTAAGTAAATAT	6636 – 6655
222	GAACAGTTAAGTAAATATGATGC	6638 – 6660
223	GTAAATATGATGCACGAAAA	6648 – 6667
224	CAAAATTACTTTGTCTGCAGAGG	6727 – 6749
225	TGCTAACCTACTGGAGGAC	6775 – 6793
226	GTTATCCCCGCCAGTGGCCACCAGCC	6805 – 5830

227	ATATAGATATGTTAGAAGCACA	6841 – 6862
228	GGAACAGCCACCAACAGAA	6880 – 6898

HPV 58

SEQ ID NO	5'→ 3'	Locus in HPV 58
229	ATGCACTGAAGTAACTAAGG	6674 – 6693
230	CACTGAAGTAACTAAGGAAG	6677 – 6696
231	TGAAGTAACTAAGGA	6680 – 6694
232	GAAGTAACTAAGGAAGGTAC	6681 – 6700
233	CTAAGGAAGGTACATATAAAAA	6688 – 6709
234	ATAAAAATGATAATTTTAAG	6703 – 6722
235	CAAAATTACACTAACTGCAGAGA	6776 – 6798
236	TTCCAATATTTTGGAGGAC	6824 – 6842
237	TTTAACACCTCCTCCGTCTGCCAGTT	6854 – 6879
238	ATATAGATTTGTTACCTCCCAG	6890 – 6911
239	AACAGCACCCCCTAAAGAA	6929 – 6947

HPV 59

SEQ ID NO	5'→ 3'	Locus in HPV 59
240	TTCTACTACTTCTTC	6643 – 6657
241	ACTACTTCTTCTATTCCTAA	6647 – 6666
242	ACTTCTTCTATTCCTAATGT	6650 – 6669
243	TCTTCTATTCCTAATGTATACAC	6653 – 6675
244	ATGTATACACACCTACCAGT	6666 – 6685
245	TAAAATAACATTAACTACAGAGG	6745 – 6767
246	TACCACTATTTTGGAGGAT	6793 – 6811
247	TGTTACACCACCTCCTACTGCTAGTT	6823 – 6848
248	ATACCGTTTTGTTCAATCTGCT	6859 – 6880
249	GGACACCGCACCGCCAGTT	6898 – 6916
250	TTATGACAAACTAAAG	6928 - 6943

HPV 61

SEQ ID NO	5'→ 3'	Locus in HPV 61
251	CTGCTACATCCCCCC	6803 – 6817
252	ACATCCCCCCCTGTATCTGA	6808 – 6827
253	CATCCCCCCCTGTATCTGAA	6809 – 6828
254	CCCCTGTATCTGAATATAAAGC	6815 – 6836

255	CTGAATATAAAGCCACAAGC	6824 – 6843
256	TAAAATACATTTAACCCCTGAAA	6903 – 6925
257	TAAGGCCTTGTTGGATGAC	6951 – 6969
258	TGTGGTACCACCACCCTCTACCAGTT	6981 – 7006
259	ATATAGGTTTTTGCAGTCCAGA	7017 – 7038
260	GGGTGCTGCTGCCCCGCCGCCC	7056 – 7077
261	CTATGCCAAGTTATCC	7089 – 7104

HPV 62

SEQ ID NO	5'→ 3'	Locus in HPV 62
262	CCGCCTCCACTGCTG	92 – 106
263	GCCTCCACTGCTGCAGCAGA	94 – 113
264	CTGCTGCAGCAGAATACACG	101 – 120
265	GCAGAATACACGGCTACCAA	109 – 128
266	CAGAATACACGGCTACCAAC	110 – 129
267	CAAAATACAGTTAACCCCCGAAA	189 – 211
268	CAAGGACCTTTTGGATGAC	237 – 255
269	GGTTTTACCTCCCCCTTCCACTAGTT	267 – 292
270	ATATCACTATTTCGAGTCTCGG	303 – 324
271	GGGGCTGCCTACCCGTCCC	342 – 360
272	GTATGCGCAAATGACA	372 – 387

HPV 66

SEQ ID NO	5'→ 3'	Locus in HPV 66
273	CAGCTAAAAGCACAT	6680 – 6694
274	CAGCTAAAAGCACATTAACT	6680 – 6699
275	CTAAAAGCACATTAACTAAA	6683 – 6702
276	TTAACTAAATATGATGCCCG	6694 – 6713
277	CTAAATATGATGCCCGTGAA	6698 – 6717
278	TAAAATAACCTTAACTGCAGAAG	6777 – 6799
279	TAATACTTTATTAGACGAT	6825 – 6843
280	CTTATCCCCACCAGTTGCAACTAGCT	6855 – 6880
281	ATATAGGTATATTAAAAGCACA	6891 – 6912
282	GGAACAGCCCCCTGCAGAA	6930 – 6948
283	CCTGGCTAAATATAAG	6960 – 6975

HPV 67

SEQ ID NO	5'→ 3'	Locus in HPV 67
284	CTGAGGAAAAATCAG	6655 – 6669
285	GAGGAAAAATCAGAGGCTAC	6657 – 6676
286	ATCAGAGGCTACATACAAAAATG	6665 – 6687
287	AGGAAAAATCAGAGGCTACA	6658 - 6677
288	CTACATACAAAAATGAAAAC	6673 – 6692
289	CAAAATATCCCTTACTGCAAATG	6752 – 6774
290	TCCAGATATATTAGAGGAC	6800 – 6818
291	CCTTACACCACCTCCTTCAGGTAATT	6830 – 6855
292	ATATAGATTTGTTACCTCGCAG	6866 – 6887
293	AACATCCCCTCCAACAGCA	6905 – 6923
294	TCTTAAAAAGTACAGT	6935 – 6950

HPV 68

SEQ ID NO	5'→ 3'	Locus in HPV 68
295	CTACTACTGAATCAG	2653 – 2667
296	TGAATCAGCTGTACCAAATA	2660 – 2679
297	GAATCAGCTGTACCAAATAT	2661 – 2680
298	CAGCTGTACCAAATATTTATGA	2665 – 2686
299	ATATTTATGATCCTAATAAA	2677 – 2696
300	TCCTGCTATTTTGGATGAT	2804 – 2822
301	TACTATAACATTGTCCACTGATG	2756 – 2778
302	TGTTGCCCCTCCACCATCTGCTAGTC	2834 – 2859
303	ATACCGCTATCTGCAATCAGCA	2870 – 2891
304	AGACGCCCCTGCACCTACT	2909 – 2927
305	ATATGATGGCTTAAAC	2939 – 2954

HPV 69

SEQ ID NO	5'→ 3'	Locus in HPV 69
306	TATTAGTACTGTATCTGCAC	6572 – 6591
307	CTGTATCTGCACAAT	6580 – 6594
308	CTGTATCTGCACAATCTGCA	6580 – 6599
309	TGCACAATCTGCATCTGCCA	6587 – 6606
310	CAATCTGCATCTGCCACTTTTA	6591 – 6612
311	CCACTTTTAAACCATCAGAT	6604 – 6623
312	TAAAATTACTCTTACCACTGATG	6683 – 6705
313	TTCTACTATTTTGGAAAAT	6731 – 6749

314	CCTTACCTTGCCTCCTACTGCTAGT T	6761 – 6786
315	ATATAGGTTTATTAAAAATTCA	6797 – 6818
316	CGATGCCCCTGCACAGCCC	6836 – 6854

HPV 70

SEQ ID NO	5'→ 3'	Locus in HPV 70
317	TGTCTGCCTGCACCGAAACG	6614 – 6633
318	CTGCACCGAAACGGC	6621 – 6635
319	GAAACGGCCATACCTGCTGT	6628 – 6647
320	CGAAACGGCCATACCTGCTG	6627 – 6646
321	CGGCCATACCTGCTGTATATAG	6632 – 6653
322	CTGTATATAGCCCTACAAAG	6644 – 6663
323	TACTATCACATTAACTGCTGACG	6723 – 6745
324	TCCTGCAATTTTGGACAAT	6771 – 6789
325	AGTTACCCCTCCACCATCTGCAAG CT	6801 – 6826
326	GTATAGGTATTTACAATCAGCA	6837 – 6858
327	GGATGCTCCTACACCTGAA	6876 – 6894
328	CTATGACGATTTAAAA	6906 – 6921

HPV 72

SEQ ID NO	5'→ 3'	Locus in HPV 72
329	ATCTGTTGGTTTAATGAGCT	6759 – 6778
330	TTTGTGACAGTTGTAGATAC	6780 – 6799
331	CTGCCACAGCGTCCT	6829 - 6843
332	ACAGCGTCCTCTGTATCAGA	6834 – 6853
333	CCACAGCGTCCTCTGTATCA	6832 – 6851
334	AGCGTCCTCTGTATCAGAATAT	6836 – 6857
335	CAGAATATACAGCTTCTAAT	6850 – 6869
336	TAAAATTCACTTAACTCCTGAAA	6929 – 6951
337	TAAGGCCTTATTGGATGAC	6977 – 6995
338	TGTGGTGCCTCCTCCTTCTACCAGTT	7007 – 7032
339	CTATAGGTTTTTGCAGTCTCGT	7043 - 7064
340	GGGGGCTGCCACCCCTCCTCCT	7082 – 7103
341	ATATGCTAACTTATCC	7115 – 7130

HPV 74

SEQ ID NO	5'→ 3'	Locus in HPV 74
342	CCTACCTCACAATCG	1686 – 1700
343	CTCACAATCGCCTTCTGCTA	1691 – 1710
344	ACCTCACAATCGCCTTCTGC	1689 – 1708
345	CAATCGCCTTCTGCTACATATA	1695 – 1716
346	ACAATCGCCTTCTGCTACATAT	1694 - 1715
347	CTACATATAATAGTTCAGAC	1708 – 1727
348	TAGTATTAAGTTAACTGCTGAGG	1787 – 1809
349	TCCTACAGTTTTAGAAGAG	1835 –1853
350	GCTAACGCCTCCCCCCAATGGTACTT	1865 – 1890
351	CTACAGATATGTGCAGTCCCAG	1901 – 1922
352	ACCTACGCCTGATAAAGCA	1940 – 1958
353	CTATGCAAATTTAAGT	1970 – 1985

HPV 82

SEQ ID NO	5'→ 3'	Locus in HPV 82
354	TGCTGTTACTCCATC	6608 – 6622
355	TGCTGTTACTCCATCTGTTG	6608 – 6627
356	ACTCCATCTGTTGCACAAAC	6615 – 6634
357	AAACATTTACTCCAGCAAAC	6631 – 6650
358	TAAAATCACTTTAACTACTGAAA	6710 – 6732
359	TTCTACAATTTTAGAACAG	6758 – 6776
360	ATTAACATTGCCCCCCTCCGCTAGTT	6788 – 6813
361	CTATCGATTTGTAAAAAATGCA	6824 – 6845
362	GGACAGTCCTCCACAGGCT	6863 – 6881

HPV CP8061

SEQ ID NO	5'→ 3'	Locus in HPV CP8061
363	TCTGTGCTACCAAAACTGTT	86 – 105
364	CTACCAAAACTGTTG	92 – 106
365	ACCAAAACTGTTGAGTCTAC	94 – 113
366	AACTGTTGAGTCTACATATAAA	99 – 120
367	GTTGAGTCTACATATAAAGC	103 – 122
368	CTACATATAAAGCCTCTAGT	110 – 129
369	TGTTATTAATTTAACAGCTGAAA	189 – 211

370	TGCTACATTACTGGAGGAC	237 – 255
371	GTTCTTACCACCTCCTACTG	267 – 286
372	CTACCGCTTTTTACAGTCTCAG	303 – 324
373	AAACAGTCCTCCTCCTGCAGAA	342 – 363
374	CTATGCAGATCTTACA	375 – 390

HPV CP8034

SEQ ID NO	5'→ 3'	Locus in HPV CP8034
375	CAGCTACATCTGCTG	92 – 106
376	GCTACATCTGCTGCTGCAGA	94 – 113
377	ACATCTGCTGCTGCAGAATACA	97 – 118
378	TGCTGCAGAATACAAGGCCT	105 – 124
379	GCTGCAGAATACAAGGCCTC	106 – 125
380	CAGAATACAAGGCCTCTAAC	110 – 129
381	TAAAATACAGTTAACACCAGAAA	189 - 211
382	CAAGGCACTGTTGGATGAT	237 – 255
383	TGTGTTGCCACCTCCTTCCACCAGTT	267 – 292
384	ATATCGCTTTTTACAGTCTCGG	303 – 324
385	GGGTGCTGCTGCCCCTGCGCCC	342 – 363
386	TTATGCCGACATGTCA	375 – 390

HPV L1AE5

SEQ ID NO	5'→ 3'	Locus in HPV L1AE5
387	ATCTACTGCAACTACTAATC	69 – 88
388	CTGCAACTACTAATC	74 – 88
389	CTGCAACTACTAATCCAGTT	74 – 93
390	ACTACTAATCCAGTTCCATCTA	79 – 100
391	CTAATCCAGTTCCATCTATA	83 – 102
392	CTATATATGAACCTTCTAAA	98 – 117
393	TAAAATTACACTTACTACTGATG	177 – 199
394	TCCTACTATTTTAGATAGT	225 – 243
395	TGTTAGTCCTCCCCCATCTGCTAGCT	255 – 280
396	ATATAGGTTTTTACAGTCATCT	291 – 312
397	GGATGTGGTTGTTCCACAA	330 – 348

HPV MM4

SEQ ID NO	5'→ 3'	Locus in HPV MM4
398	CTGCTGTTACTCAATCTGTT	92 – 111
399	TGCTGTTACTCAATC	93 – 107
400	GTTACTCAATCTGTTGCACA	97 – 116
401	TGCACAAACATTTACTCCAG	111 – 130
402	TTACTCAATCTGTTGCACAAAC	98 – 119
403	AAACATTTACTCCAGCAAAC	116 – 135
404	TAAAATCACTTTAACTACTGAAA	195 – 217
405	TTCTACAATTTTAGAACAG	243 – 261
406	ATTAACCTTGCCCCCCTCAGCTAGTT	273 – 298
407	CTATCGATTTGTAAAAAATGCA	309 – 330
408	GGACAGTCCTCCACAGGCT	348 – 366

HPV MM7

SEQ ID NO	5'→ 3'	Locus in HPV MM7
409	TGCTGCTACACAGGC	93 – 107
410	GCTGCTACACAGGCTAATGA	94 – 113
411	TGCTACACAGGCTAATGAAT	96 – 115
412	CTACACAGGCTAATGAATACAC	98 – 119
413	ATGAATACACAGCCTCTAAC	110 – 129
414	CAAAATACATCTTACCCCTGAAA	189 – 211
415	TGAACATTTATTGGATGAG	237 – 255
416	CGTGTTACCACCTCCTTCCACCAGCC	267 – 292
417	CTATCGCTATCTGCAGTCCCGT	303 – 324
418	GGGTCCTTCCGCCCCTGCCCCT	342 – 363
419	TTATGATGGCCTTGTA	375 – 390

HPV MM8

SEQ ID NO	5'→ 3'	Locus in HPV MM8
420	TGCTACCAACACCGA	93 –107
421	CTACCAACACCGAATCAGAA	95 –114
422	CCAACACCGAATCAGAATATAA	98 –119
423	CAGAATATAAACCTACCAAT	110 – 129

424	TAAGGTCCGTCTGACTCCAGAGG	189 – 211
425	TGACTCCTTATTAGATGAG	237 – 255
426	TGTTGTGCCCCCTCCCTCCACAAGTT	267 – 292
427	CTATAGGTACTTGCAGTCTCGC	303 – 324
428	GGGGGCCGCCGCCGCCAAGCCT	342 – 363
429	TTATGCTGGCATGTCC	375 – 390

[0088] The sequences of the probes listed above are either identical or complementary to the corresponding sequences of HPV subtypes so that the probes can hybridize with the sequences of HPV subtypes perfectly.

[0089] According to a preferred embodiment of the present invention, a detector for detecting and simultaneously diagnosing 39 subtypes of human papilloma viruses (HPV) contained in a biological sample is provided. Please refer to Fig. 1. The detector 10 is an oligonucleotide biochip. The detector 10 includes a carrier 11 and a plurality of micro-dots 12 immobilized on the carrier 11. The carrier 11 is a nylon membrane. Each micro-dot 12 is used for identifying one particular HPV subtype. There is at least one oligonucleotide sequence contained in each micro-dot 12 that is specific to one particular HPV subtype. The oligonucleotide sequences are the probes selected from the above list for each HPV subtype respectively. For example, the probe on the carrier 11 could contain at least one sequence, which is selected from SEQ ID NO 1 to SEQ ID NO 12 (shown above), for identifying the subtype 6 of human papilloma viruses (HPV 6).

[0090] As described in the above, the probes will hybridize specifically with the L1 gene sequence of the corresponding HPV subtype. Preferably, the probes have a length between 15-30 bases. The oligonucleotide sequences contained in each micro-dot 12 serve as a detection probe, which hybridizes

specifically with the L1 gene sequence of the particular HPV subtype to form a hybridization complex as a detection indicator. Therefore, each micro-dot 12 identifies a specific HPV subtype via a corresponding oligonucleotide of the specific HPV subtype, and thereby detecting and simultaneously identifying subtypes of human papilloma viruses. The sequences of the oligonucleotides provided by the present invention are specific to the epidemics of human papilloma viruses. The detector 10 is able to simultaneously identify 39 different HPV subtype that are HPV 6, HPV 11, HPV 16, HPV 18, HPV 26, HPV 31, HPV 32, HPV 33, HPV 35, HPV 37, HPV 39, HPV 42, HPV 43, HPV 44, HPV 45, HPV 51, HPV 52, HPV 53, HPV 54, HPV 55, HPV 56, HPV 58, HPV 59, HPV 61, HPV 62, HPV 66, HPV 67, HPV 68, HPV 69, HPV 70, HPV 72, HPV 74, HPV 82, HPV CP8061, HPV CP8034, HPV L1AE5, HPV MM4, HPV MM7 and HPV MM8. Furthermore, the detector 10 includes the micro-dot 12 containing a Glutaldehyde-3-phosphodehydrogenase (GAPDH) gene, which is used as an internal control.

[0091] EXAMPLE I

The method for immobilizing or mounting the above mentioned probes (oligonucleotides) on the carrier 11 (the nylon membrane) is described as follows.

[0092] 1. -TTTTTTTTTTTTTTT (SEQ ID NO 469) is added to the 3' end of the oligonucleotide provided by the present invention by terminal transferase according to the following steps 1.1 to 1.3.

1.1 Mixing the following components:

10X NEBuffer 4	5 μl
2.5 mM $CoCl_2$	5 μl

oligonucleotide	5 ~ 300 pmol
10 ~ 300 mM dATP、dCTP、dTTP or dGTP	1 μl
Terminal Transferase (20U/μl) (NEW English BioLabs,M0252S)	0.5 ~ 5 μl
Add M.Q. H_2O to final volume	50 μl

1.2 The components are mixed at 37℃ for 15~60 minutes.

1.3 10 μl of 0.2 M EDTA (pH 8.0) is added to the mixture to stop the reaction.

[0093] 2. The oligonucleotide having 3' end labeling is mounted on the carrier 11 according to the following steps 2.1 to 2.3.

2.1 The oligonucleotide having 3' end labeling is mounted on the carrier 11 by a needle having a 400 μm wide head. The distance between each dot is 1200 μm.

2.2 The carrier 11 having the dot array 12 thereon is exposed to UV light, and the detector 10 is formed.

2.3 The detector 10 is preserved in a drying box.

[0094] EXAMPLE II

According to another preferred embodiment of the present invention, the carrier 11 could be a glass plate. The method for immobilizing or mounting the above mentioned probes (oligonucleotides) on the carrier 11 (glass plate) is described as follows.

[0095] 1. The surface of the carrier 11 is treated according to the following steps 1.1 to 1.8.

1.1 The carrier 11 is cleaned in non-fluorescent and soft cleaner.

1.2 The clean carrier 11 is immersed in 10% NaOH.

1.3 The carrier 11 is oscillated in double-distilled water, 1% HCl solution and methanol in sequence for 2 minutes, and dried in an oven.

1.4 The carrier 11 is immersed in 1% 3-aminopropyltrimethoxysilane (APTMS) in 95% aqueous acetone at room temperature for about 2 minutes.

1.5 The carrier 11 is washed in acetone, and the carrier 11 is dried in the oven at 110℃ for 45 minutes.

1.6 The dried carrier 11 is immersed in 0.2% 1,4-phenylene diisothiocyanate, wherein the solvent is 10% pyridine in dimethyl formamide), at room temperature for 2 hours.

1.7 The carrier 11 is washed in methanol and acetone, and then the carrier 11 is dried.

1.8 The dried carrier 11 is preserved in a vacuum and dry box.

[0096] 2. The oligonucleotides provided by the present invention are mounted on the carrier 11 (the glass plate) according to the following steps 2.1 to 2.3.

2.1 The oligonucleotide having 3' end labeling is mounted on the carrier 11 by a needle having a 400 μm wide head. The distance between each dot is 1200 μm.

2.2 The carrier 11 is immersed in 1% NH_4OH solution for about 2 minutes, washed in double-distilled water, and then dried at room temperature. Thus, the detector 10 is formed.

2.3 The detector 10 is preserved in a dried box.

[0097] According to the above description, a biochip for specifically identifying the subtypes of human papilloma viruses contained in a biological sample is provided. Please refer to Fig. 2(a). The biochip 20 includes a carrier 21 and a plurality of micro-dots 22 immobilized on the carrier 21. The

carrier 21 is a nylon membrane. The actual length of the nylon membrane is about 1.44 cm and the actual width of the nylon membrane is about 0.96 cm. The micro-dots 22 are mounted on the carrier 21 according to the foresaid method, wherein the distance between each dot is about 1.2 mm and the diameter of each dot is about 0.4 mm. Each micro-dot 22 contains at least one oligonucleotide. (15~30mer), and each micro-dot 22 is used for specifically identifying a specific HPV subtype. The sequence of the oligonucleotide is selected from the foresaid list.

[0098] The subtype of human papilloma viruses identified by each dot of the micro-dots 22 is illustrated in Fig. 2(b). SC (system control) presents the PCR product amplified from any subtype of human papilloma viruses and biotin-contained primer. NC (negative control) presents the plants DNA fragment irrelevant to HPV. IN (internal control) presents the sequence 5'-gcccagactgtgggtggcag-3' (SEQ ID NO 470) of the housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). In sum, the biochip 20 provided in the present invention is able to detect and simultaneously identify 39 different HPV subtypes contained in the biological sample.

[0099] According to another preferred embodiment of the present invention, a method for detecting and simultaneously diagnosing 39 subtypes of human papilloma viruses (HPV) contained in a biological sample is provided. The steps are generally described as follows. First, the L1 gene fragment of human papilloma viruses (HPV) contained in the biological sample is amplified by polymerase chain reaction (PCR) using primers labeled with signaling substance. After the amplification product is obtained, it is hybridized with the detector 11 as describe above to form a hybridization complex. Then, the nonhybridized amplification product is removed from the detector 11. Next,

the detector 11 is detected for the existence of the hybridization complex through detecting the signaling substance. The micro-dot 12 having the signaling substance shown thereon means a positive result that the biological sample contains the specific HPV subtypes recognized by the corresponding micro-dot 12. Ultimately, the HPV subtypes contained in the biological sample are thereby detected and simultaneously identified.

[00100] The method provided by the present invention for detecting and simultaneously identifying 39 subtypes of human papilloma viruses contained in a sample is described as follows.

[00101] EXAMPLE III

1. The biological sample obtained from the patient is treated according to the following steps 1.1 to 1.3.

1.1 The cells are centrifuged at 1,500 rpm at 20℃ for 5 minutes.

1.2 The cell pellet is washed in 10 mM Tris (pH 8.5) and dissolved in 8 mM NaOH. Then, the solution is transfer to 1.5 mL micro-tube.

1.3 A proper amount of TreTaq (1U/μl) solution is added to the micro-tube. The reaction is carried out at 95℃ for 1 hour. The DNA contained in the sample is obtained after centrifugation at 13,500 rpm, 20℃ for 5 minutes. The otained DNA is preserved at -20℃.

[00102] EXAMPLE IV

2 The L1 gene fragment of human papilloma viruses (HPV) contained in the biological sample is then amplified by polymerase chain reaction (PCR). The polymerase chain reactions are performed according to the following steps.

[00103] 2.1 Glutaldehyde-3-phosphodehydrogenase (GAPDH) gene is used as the internal control of the polymerase chain reactions so that it could help

confirm whether the detecting protocols are precisely followed. The steps are described according to the following steps 2.1.1 to 2.1.3.

2.1.1 Mixing the following components:

Reagent	Stock	amount	Final concentration
Sterile H_2O		2.6	
10X *Taq* Buffer		0.5	1X *Taq* Buffer
dNTP	2.5 mM	0.4	200 μM
Template		1	
GAP241-5[1)] primer	10 pmol/μl	0.2	0.4 pmol/μl
GAP241-3[2)] primer	10 pmol/μl	0.2	0.4 pmol/μl
ProTaq (PROTECH)	5 U/μl	0.1	0.1 U/μl
Total volume (μl)		5	

1) Gap241-5 (SEQ ID NO 471): CCACCAACTGCTTAGCACCCC

2) Gap241-3 (SEQ ID NO 472): TGCAGCGTACTCCCCACATCA

3) The proper amount of mineral oil is added to prevent the evaporation.

2.1.2 The polymerase chain reaction is performed according to the following programs.

Program 1	Program 2	Program 3
	94℃ , 15 seconds	
94℃ ,	57℃ ,	72℃ ,
3 minutes	1 minute	5 minutes
	72℃ , 30 seconds	
	40 cycles	

2.1.3 The product of the polymerase chain reaction is analyzed in 2.5% agarose/EtBr (0.5×TBE).

[00104] 2.2 The DNA contained in the sample is amplified by the polymerase chain reaction according to the following steps.

2.2.1 Mixing the following components:

Reagent	Stock	Amount	Final concentration
Sterile H_2O		4.7-5.7	
10X *Taq* Buffer		1	1X *Taq* Buffer
dNTP	2.5 mM	0.8	200 μM
Template		1-2	
BSA	10 mg/ml	0.1	0.1 μg/μl
Primer[1,2)]	10 pmol/μl	0.6	0.6 pmol/μl
Primer[1,2)]	10 pmol/μl	0.6	0.6 pmol/μl
ProTaq (PRO_{TECH})	5 U/μl	0.2	0.1 U/μl
Total volume (μl)		10	

1) MY09/MY11: Weimin et al., 1997, J. Clin. Microbiol. 35(6): 1304-1310

2) MY11/GP6+: Weimin et al., 1997, J. Clin. Microbiol. 35(6): 1304-1310

3) The proper amount of mineral oil is added to prevent the evaporation.

4) The 5' end of the MY09 and GP6+ primers could be labeled with biotin or Cy5 fluorescent substances.

2.2.2 The polymerase chain reaction is performed according to the following programs.

Program 1	Program 2	Program 3

	94℃ , 45 seconds	
94℃ ,	45℃ ,	72℃ ,
3 minutes	1 minute	5 minutes
	72℃ , 1.5 minutes	
	45 cycles	

2.2.3 The product of the polymerase chain reaction is analyzed in 2.5% agarose/EtBr (0.5×TBE).

[00105] According to the above description, the biochip 20 is used for identifying different HPV subtypes. In one embodiment of the invention, the positive clones of human papilloma viruses are used and detected according to the foresaid method. As previously mentioned, the PCR amplification product could be obtained by different primer sets. One is primer set MY09/MY11, the other is primer set MY11/GP6+. Therefore, the positive clones are respectively amplified by PCR using MY11/MY09 primers and MY11/GP6+ primers. The products of the polymerase chain reaction are analyzed in 2.5% agarose/EtBr, and the electrophoresis results are shown in Fig. 3(a)-(c). Fig. 3(a) shows the electrophoresis result of the analyzed PCR products using primer set MY09/MY11. In Fig. 3(a), M presents DNA marker. Lane 1~20 present HPV 6, HPV 11, HPV 16, HPV 18, HPV 26, HPV 31, HPV 33, HPV 35, HPV 44, HPV 45, HPV 52, HPV 53, HPV 54, HPV 56, HPV 59, HPV 61, HPV 66, HPV 70, HPV CP8061, and HPV L1AE5 in sequence. Fig. 3(b) shows the electrophoresis result of the analyzed PCR products using primer set MY11/GP6+. In Fig. 3(b), M presents DNA marker. Lane 1~39 present HPV 6, 11, 16,18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 56,

58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061, CP8304, L1AE5, MM4, MM7, and MM8 in sequence. Fig. 3(c) shows the electrophoresis result of the PCR products using GAPDH primer set. Clearly, the electrophoresis results show the PCR products with correct sizes. That is, PCR products using primer set MY09/MY11 is about 450 bp, the PCR products using primer set MY11/GP6+ is about 190 bp, and the PCR products using GAPDH primer set is about 190 bp.

[00106] EXAMPLE V

3. When the carrier 11 is a nylon membrane, the detector 10 provided by the present invention is used for identifying the subtypes of human papilloma viruses according to the following hybridization steps.

3.1 The detector 10 is immersed in 2x SSC solution for 5 minutes.

3.2 The detector 10 is immersed in a buffer containing salmon sperm DNA (50 μg/μl), and the oligonucleotides mounted on the detector 10 are pre-hybridized with the salmon sperm DNA at 35℃ for 30 minutes.

3.3 The PCR product having biotin labeled thereon is added into and mixed with a buffer containing salmon sperm DNA (50 μg/μl) at 95℃ for about 5 minutes. The denatured DNA is placed on ice.

3.4 The denature DNA is added to the detector 10 and hybridized with the oligonucleotides at 35℃ for 4 hours or overnight.

3.5 The detector 10 is washed in 2x SSC/1% SDS solution at 35℃ for 15 minutes.

3.6 The detector 10 is washed in 0.2x SSC/0.1% SDS solution at 35℃ for 15 minutes.

3.7 The detector 10 is treated in 0.5% isolation reagent for 1 hour.

3.8 The detector 10 is treated with avidin-alkalinephosphatase for about 1 hour.

3.9 The detector 10 is washed in 1x PBST solution.

3.10 The detector 10 is washed in Tris/NaCl solution.

3.11 The detector 10 is treated with NBT/BCIP at room temperature to show the reacting dot in blue.

3.12 The blue dot having the specific oligonucleotide sequence presents the specific subtype of human papilloma viruses contained in the sample.

[00107] Preferably, the foresaid PCR amplified products shown in Figs. 3(a)and 3(b) are then respectively detected by the biochip 20 according to the above steps and the results are shown in Figs. 4(a) and 4(b). Fig. 4(a) shows the detecting result of detecting the PCR products using primer set MY09/MY11 of HPV positive clones. Fig. 4(b) shows the detecting result of detecting the PCR products using primer set MY11/GP6+ of HPV positive clones. When comparing the results shown in Fig. 4(a) and Fig. 3(b) based on the "SC" dot, it is very clear that the biochip 20 can precisely identify the subtype of human papilloma viruses. Take the result of HPV 6 as example. Since this biochip is hybridized with the PCR product amplified from HPV 6 positive clone, there should be 6 positive micro-dots shown on the biochip 20, including 2 SC micro-dots at the corners, 2 SC micro-dots in the central, and 2 micro-dots of HPV 6. The result clearly shows the exact 6 positive micro-dots without any other false positive micro-dot. Obviously, all the results of other biochips in Figs. 4(a) and 4(b) show a clear and clean result as well. In other words, there is no cross reaction occurred in the detection, which proves that the biochip provided in the present invention has a very high specificity.

[00108] In addition, in another embodiment of the invention, the biological sample obtained from the patient is used and detected. The biochip 20 and the detection method described in the above are used for detecting and identifying the HPV subtypes contained in the sample according to the foresaid method. The results are shown in Fig. 5. When comparing the results shown in Fig. 5 and Fig. 3(b) based on the "SC" dot, the results show that HPV 53 is contained in the sample (1), HPV 45 is contained in the sample (2), HPV 52 is contained in the sample (3), and HPV 39 is contained in the sample (4). Therefore, when detecting the biological sample obtained from a patient, it is very clear that the biochip 20 can precisely identify the subtype of human papilloma viruses.

[00109] EXAMPLE VI

According to another embodiment of the present invention, the carrier 11 could be a glass plate. When the carrier 11 is a glass plate, the detector 10 provided by the present invention is used for identifying the subtypes of human papilloma viruses according to the following hybridization steps.

4.1 The PCR product having Cy5 labeled thereon is purified by PCR Clean Up-M System (Viogene, USA), and the PCR product is precipitated in ethanol. Then, the PCR product is dried.

4.2 The precipitated DNA is dissolved in 12 μl of the buffer (2x SSC/0.1% SDS), and centrifugated for 1 minute, and then placed on boiled water for 2 minutes. Then, the mixture is placed on ice for 5 minutes.

4.3 The mixture is centrifugated for 30 seconds, and 10 μl of the mixture is added to the left side of the dot array 22. A cover slice is carefully covered on the dot array from the left side of the dot array to prevent the bubble formation.

Then, the detector 10 is place in Humid Chamber (Sigma, USA), and the dot array is faces downward at 35℃ for 4 hours or overnight.

4.4 The detector 10 is vertically placed in the solution A (2x SSC/1% SDS), and the detector is slightly oscillated apart from the cover slice. Then, the detector 20 is washed in a shaker at 160 rpm for 12 minutes.

4.5 The detector 10 is washed in the solution B (0.2x SSC/0.1% SDS) and oscillated at 35℃ for 12 minutes. The detector 10 is washed in water. Then the detector 10 is dried.

4.6 The dried detector 10 is scanned by GenePix™4000 (Axon, USA), excited by the light having 635 nm of wavelength, and analyzed by GenePixPro 3.0 (Axon, USA).

[00110] According to the above description, a biochip for specifically identifying the subtypes of human papilloma viruses contained in a biological sample is provided. Please refer to Figs. 6(a) and (b). The biochip 30 includes a carrier 31 and a plurality of micro-dots 32 immobilized on the carrier 31. The carrier 31 is a glass plate. The micro-dots 32 are immobilized on the glass plate 31 according to the foresaid method. Each micro-dot 32 contains at least one oligonucleotide (15~30mer), and each micro-dot 32 is used for specifically identifying a specific HPV subtype. The sequence of the oligonucleotide is selected from the foresaid list. The subtype of human papilloma viruses identified by each dot of the micro-dots 32 is illustrated in Fig. 6(b).

[00111] The biochip 30 is stained with SYBR Green II, scanned by GenePix™ 4000 (Axon, USA) and excited by the light having 635 nm of wavelength. The result is shown in Fig. 7(a). Preferably, the foresaid PCR amplified products are then detected by the biochip 30 according to the above

steps and the results are shown in Figs. 7(b). When comparing the results shown in Fig. 7(a) and Fig. 6(b), it is very clear that the biochip 30 can precisely identify the subtype of human papilloma viruses. The result clearly shows the exact positive micro-dots without any other false positive micro-dot. Besides, there is no cross reaction occurred in the detection, which proves that the biochip provided in the present invention has a very high specificity. Therefore, the biochip having different carriers (made of nylon membrane or glass plate) can obtain the same results and same specificities.

[00112] According to the above, the drawbacks in the conventional HPV detecting kit do not exist in the HPV detecting kit provided in the present invention. The HPV detecting kit of the present invention is able to diagnose multiple HPV subtypes (up to 39 different subtypes) at the same time, allowing the rapid and reliable detection and identification of HPV possibly present in a biological sample. Besides, an internal control is included in the detector to show whether the detecting process is well handled so that the detecting result is dependable. In addition, HPV detecting kit of the present invention has a high specificity and accuracy. Hence, the present invention not only has a novelty and a progressive nature, but also has an industry utility.

[00113] While the invention has been described in terms of what is presently considered to be the most practical and preferred embodiments, it is to be understood that the invention needs not be limited to the disclosed embodiments. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and scope of the appended claims which are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures.